PRECLINICAL EVIDENCE FOR THE EFFICACY OF ANGIOTENSIN RECEPTOR ANTAGONISM IN A RODENT MODEL OF VULNERABILITY TO COMORBID DEPRESSION AND CARDIOVASCULAR DISEASE

by

Kristen A. Stedenfeld

B.A. in Psychology, New York University, 2004

Submitted to the Graduate Faculty of

Arts and Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2011

UNIVERSITY OF PITTSBURGH

COLLEGE OF ARTS AND SCIENCES

This dissertation was presented

by

Kristen A. Stedenfeld

It was defended on

June 7, 2011

and approved by

Peter J. Gianaros, PhD, Associate Professor, Department of Psychiatry

Linda Rinaman, PhD, Professor, Department of Neuroscience

Susan R. Sesack, PhD, Professor, Department of Neuroscience

Etienne L. Sibille, Associate Professor, Department of Psychiatry

Dissertation Advisor: Alan F. Sved, Professor and Chairman, Department of Neuroscience

PRECLINICAL EVIDENCE FOR THE EFFICACY OF ANGIOTENSIN RECEPTOR ANTAGONISM IN A RODENT MODEL OF VULNERABILITY TO COMORBID DEPRESSION AND CARDIOVASCULAR DISEASE

Kristen A. Stedenfeld, PhD

University of Pittsburgh, 2011

Major depressive disorder and cardiovascular disease are highly comorbid, and the presence of one disorder greatly increases the likelihood of the other. Remarkably, depressed but otherwise healthy patients with no history of cardiovascular disease are as likely to have a heart attack as patients with established cardiovascular disease.

Experimental studies have used chronic mild stress (CMS), a rodent model of depression that uses a series of unpredictable, intermittent, and variable mild stressors to induce anhedonia, one of the core diagnostic criteria for major depression. CMS also induces a constellation of behavioral, physiological, and neuroendocrine responses that closely resemble those observed in depressed patients, including alterations in autonomic control of the heart marked by decreased heart rate variability (HRV). Commonly prescribed antidepressants might not improve cardiovascular alterations associated with depression, even when depressive signs are ameliorated. There is evidence, however, that candesartan, an angiotensin type 1 receptor (AT1R) antagonist (ARB) often prescribed for cardiovascular disorders, has anxiolytic and possibly antidepressant effects in animal models.

To study the possible antidepressant effects of candesartan, we first established a robust rodent model of vulnerability to depression, since severity of depression is correlated with

severity of cardiovascular changes in humans. We found that rats selectively-bred for low locomotor responses to a novel environment (bLR) were especially vulnerable to CMS-induced anhedonia and cardiovascular changes. Conversely, selectively-bred high-responder rats (bHR) were resilient to the behavioral and cardiovascular changes induced by CMS.

Finally, we compared the effects of candesartan and the SSRI fluoxetine on CMS-induced anhedonia and cardiovascular changes. We found that candesartan has profound antidepressant effects, including rapid reversal of anhedonia, and attenuated anxiety-like behavior. Furthermore, candesartan reversed cardiovascular changes, including clinically relevant markers of risk for cardiac mortality. Thus the major findings of these studies are twofold: (1) bHR/bLR rats exposed to CMS offer a robust model of the interactions of predisposition and environmental stress that may contribute to depression and comorbid cardiovascular disease and (2) candesartan and other ARBs may be novel therapies for the treatment of comorbid depression and cardiovascular disease, and may be more effective than traditionally-prescribed antidepressants such as SSRIs.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

The completion of these experiments was only possible due to the efforts of a large and diverse group of supportive and hard-working people. I hope that anyone reading this section will forgive me for its length and occasional sentimentality. When it comes to the expression of gratitude, I feel that it's best not to be stingy.

I am so grateful for the opportunities given to me by the Department of Neuroscience, the Center for Neuroscience at the University of Pittsburgh, and the Center for the Neural Basis of Cognition. It is inspiring to be surrounded by so many wonderful, smart, passionate people.

Boundless thanks go to my advisor and mentor, Dr. Alan Sved. Alan is a thoughtful mentor, an inspiring teacher and an awesome scientist. I would like to thank Alan for his generosity in encouraging my projects, his commonsense approach to experiments, his patience with my often inconvenient schedule, for always pushing me to be a better, more careful scientist, for imparting both his knowledge and his passion for science, and most of all for his kindness.

Many thanks go to my wonderful committee members for their support, encouragement warmth, and enthusiasm. Thank you to Dr. Etienne Sibille, the chair of my committee, for his practical and straightforward outlook. Thank you to Dr. Susan Sesack for her humor and candor. Thank you to Dr. Peter Gianaros for his advice and guidance during my adventure with heart rate variability. And special thanks go to Dr. Linda Rinaman, who was the first person to show me

how to pick up a rat during my rotation in her lab, and who has been supportive ever since. I would also like to thank Dr. Randall Sakai, my outside examiner, for adding another unique voice to my diverse committee. Thanks also to Dr. Ed Stricker and Dr. Pat Card, former committee members who have each been powerful sources of support and guidance at critical points in my graduate career.

I am so thankful for the friendly encouragement of Joan Blaney and Marlene Nieri, and for the staff of the Department of Neuroscience fiscal office who keep the rest of us going: Mary Spanoudakis, Debbie Glumac, Kathy Short, and Joni Mainier. They are an impressive group of women who do so much and yet always find the time to say hello.

I would like to thank my collaborators, Dr. Ilan Kerman and Dr. Sarah Clinton from the University of Alabama Birmingham, and Dr. Huda Akil and Dr. Stan Watson from the University of Michigan, who supplied me with the selectively-bred strains of rats used in these studies. I absolutely could not have done these experiments without them! I am especially thankful to Ilan and Sarah who have been such great role models for me as I figure out how to balance work and science and parenthood. I would also like to thank Dr. Angela Grippo, who pioneered this line of research. Angela's publications were the inspiration for these projects, and she was always willing to share her knowledge and encourage me along the way. The research of Dr. Juan Saavedra is the other half of the inspiration behind these experiments, and I am grateful and humbled at the opportunity to collaborate with him.

Thank you to Dr. Israel Christie for his tutelage in the fine art of frequency domain analysis, and the use of his fantastic software, *Physioscripts*. Without his help, I would still be sorting through an unending pile of numbers. Thank you to Dr. Debbie Chapman and Dr. Joe Newsome who went out of their way to find a way for me to do these experiments in our animal

facility. Thank you to Barb Cohen and Michelle Reddy for caring for my rats and making accommodations for these sometimes-strange experiments.

Many thanks to the members of the Sved lab including Xia Li who taught me to be careful with brains, and Dr. Judy Sved who taught me excellent surgical technique. Thanks also to Dr. Gina Cano and Vivian Allahyari, for their camaraderie and encouragement. I was also fortunate to have the help of two outstanding undergraduate students, Nichole Johnson and Jenna Parrish, who always kept me laughing and reminded me how fun and strange science can be.

I am especially grateful for the help, support, and friendship of Myriam Stricker, who entered my life as a coworker and quickly became one of my most treasured friends. There is not a single area of my life that Myriam's generosity and kindness has left untouched, and I am a better person because of her friendship. I could not have completed these experiments or finished my dissertation without her considerable help, nor would the process have been nearly as enjoyable. Myriam, from the bottom of my heart: Thank you.

A number of dear friends have made my graduate experience a truly wonderful one, including (but not limited to) Dr. Kate Cosgrove, Dr. Ericka Holmstrand, Jesse Wood, and Joy Balcita-Pedicino. An extra special bit of gratitude is owed to Dr. Layla Banihashemi, who has been such a fabulous friend and supporter from my very first day of graduate school. Thank you, Layla, for always listening.

I am so blessed to be buoyed by the love of an extraordinary family, including my parents, Karen and Randy Maynard and Dennis Stedenfeld. My mother, Karen, deserves much of the credit for nurturing my curiosity, my fascination with taking things apart to figure out how they work, and my admiration of the resilience of the human spirit. Warm thanks go to my incredible sister, Laura Stedenfeld, for her steadfast encouragement and support. I would also

like to acknowledge the profound influence of my late grandfather, Arthur O'Brien, who was the first person to show me how science could explain the things in nature I loved so much. Many thanks to my family-in-law, who have adopted me as one of their own from the beginning (whether I liked it or not): Joseph Schmich, Tina Schmich and Lori Nelson, Meredith Schmich, and Dustin Schmich. You are all a bunch of lunatics, and I love you.

In closing, I would like to express my tremendous love and gratitude for my husband Ben, my partner in all things, closest friend, and all-around favorite person. I honestly don't know what I would do without him. And last, but far from least, thank you to my amazing daughter, Adelaide. Every day that I spend with her I'm reminded of what a great big beautiful world we live in. I am so very lucky to have been able to spend my time studying one small corner of it.

LIST OF ABBREVIATIONS

5-HTT – Serotonin transporter

ACE – Angiotensin converting enzyme

ANS – Autonomic nervous system

ARB – Angiotensin II type 1 receptor blocker

AT1Rs – Angiotensin type 1 receptors

bHR – Selectively-bred high-responder rat

bLR – Selectively-bred low-responder

BP – Blood pressure

BrdU - 5-bromo-2'-deoxyuridine

CMS – Chronic mild stress

CRH – Corticotrophin-releasing hormone

DSM-IV – Diagnostic and statistical manual of mental disorders, 4th edition

EPMZ – Elevated plus maze

FRL – Flinders resistant line

FSL – Flinders sensitive line

FST – Forced swim test

HAB – High anxiety-like behavior rat

HR – Heart Rate

HiR – High-responder rat (supplier-bred)

HPA – Hypothalamic pituitary adrenal

HF – High frequency

HRV – Heart rate variability

IBI – Inter-beat interval

IL-1β – Interleukin 1β

IL-6 – Interleukin-6

LAB – Low anxiety-like behavior

LF – Low frequency

LoR – Low-responder rat (supplier-bred)

NSF – Novelty-suppressed feeding

PVN – paraventricular nucleus of the hypothalamus

RAS – Renin angiotensin system

SA – Sinoatrial

SEM – Standard error of the mean

SPT – Sucrose preference test

SSRI – Selective serotonin reuptake inhibitor

TNF-α - Tumor necrosis factor - α

TP – Total power

VLF – Very low frequency

1.0 INTRODUCTION

1.1 DEPRESSION AND CARDIOVASCULAR DISEASE ARE HIGHLY COMORBID

Emotions, personality, and mood can have a tremendous impact on human physiology, and prolonged periods of emotional distress can have serious repercussions on long-term health. A profound example of this interaction of psychology and physiology is the highly comorbid relationship between major depressive disorder and cardiovascular disease. Incredibly, depressed but otherwise healthy patients with no history of cardiovascular disease are as likely to have a heart attack as patients with established cardiovascular disease [1, 2]. This relationship exists independent of common risk factors, such as increased body mass index, smoking, or preexisting cardiac pathophysiology [3]. Furthermore, depression is considered a significant risk factor for coronary heart disease and cardiac mortality [2-4]. In the six months following a myocardial infarction, mortality was 3.5 times higher in patients who were also depressed than those who were not [2]. The association between depression and cardiovascular disease is also bidirectional, and patients who have recently had a major cardiac event, such as a myocardial infarction are more likely to be diagnosed with depression. Indeed, while approximately 2-9% of American adults suffer from depression, the prevalence of this disorder in patients recovering from myocardial infarction is between 20-40% [5-7]. Cardiovascular disease and major depression are currently two of the most detrimental disorders in developed countries and

prevalence of both disorders continues to rise [8, 9]. Although the comorbid, bidirectional relationship between depression and cardiovascular disease is gaining attention [10], many important questions regarding the relationship between the two disorders, and the mechanisms that might connect them, remain unanswered.

1.2 MAJOR DEPRESSION: A DISORDER OF MOOD AND PHYSIOLOGY

1.2.1 Diagnostic criteria for depression

Major depressive disorder is a complex and devastating disorder with significant behavioral, physiological, and neuroendocrine alterations. It is estimated that somewhere between 20 - 25% of the population will suffer from some form of depression in their lifetime [11]. The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) lists two main criteria of the disorder: persistent depressed mood, and anhedonia, a lack of or reduction in pleasure for things that used to be pleasurable. A clinical diagnosis of major depression requires either both of these criteria or one of these criteria accompanied by a certain number of the following changes: changes in body weight or appetite, changes in sleep pattern, low energy or fatigue, diminished concentration, feelings of worthlessness, guilt, or despair, or recurrent thoughts of death or suicide [12] (**Figure 1**).

DSM-IV Criteria for Major Depressive Episode
Five (or more) of the following symptoms present during the same 2-week period; at least one symptom is (1) or (2)
(1) depressed mood most of the day, nearly every day (2) markedly diminished interest or pleasure in all, or almost all, activities
(3) significant weight loss or weight gain, or decrease or increase in appetite (4) insomnia or hypersomnia (5) psychomotor agitation or retardation (6) fatigue or loss of energy (7) feelings of worthlessness or excessive or inappropriate guilt (8) diminished ability to think or concentrate, or indecisiveness (9) recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

Figure 1: Diagnostic criteria for major depressive disorder, from the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV).

1.2.2 Physiological changes that co-occur with depression

In addition to these diagnostic criteria, a number of neuroendocrine and physiological changes have been observed in depressed patients: increased levels of sympathetic activity, marked by increased norepinephrine excretion [13], hyperactivation of the hypothalamic pituitary adrenal (HPA) axis [14-17], increased levels of immune factors, such as pro-inflammatory cytokines [18], and cardiovascular changes, such as increased resting heart rate (HR) and decreased heart rate variability (HRV) [19-21] . Decreased HRV is an especially critical symptom of depression, as it is also used as a clinical marker for increased risk of cardiac mortality [22], and will be discussed in detail below.

1.3 AUTONOMIC CONTROL OF THE HEART IS ALTERED IN DEPRESSION AND CARDIOVASCULAR DISEASE

1.3.1 Normal autonomic control of the heart

Heart rate is controlled by the sinoatrial (SA) node, a cluster of autorhythmic cells located in the right atrium of the heart, which generates electrical impulses that cause the heart to contract. The SA node is often called the pacemaker of the heart, and receives neural input from both complementary branches of the autonomic nervous system (ANS). The sympathetic branch of the ANS, also called the “fight or flight” branch has excitatory effects on the periphery, working to mobilize the body to deal with the source of stress: blood flow to muscles increases, digestion

is inhibited, and HR is increased. The parasympathetic branch, sometimes called the "rest and digest" branch, is generally inhibitory, helping to return the body to rest after a stress, decreasing HR, and aiding in activities such as digestion, sexual arousal, and growth. These dual branches of the ANS work together to modulate heart rate based on the state of the organism. At rest, both branches are active, though the parasympathetic branch predominates to keeps HR at a rate that is lower than intrinsic SA node activity. This is sometimes referred to as a parasympathetic "brake" on HR, and allows an extra level of control over HR. In times of stress, HR can be increased by inhibiting parasympathetic activity and increasing sympathetic activity; conversely, HR is decreased by increasing parasympathetic and inhibiting sympathetic activity. The sympathetic and parasympathetic branches of the ANS are each controlled by brainstem areas of the central nervous system (**Figure 2**). Pre-sympathetic neurons in the brainstem (e.g. rostral ventrolateral medulla and raphe pallidus) project to the intermediolateral cell column in the spinal cord, which project in turn to the sympathetic ganglia that innervate the heart. The parasympathetic (i.e. vagal) branch originates in the nucleus ambiguus and dorsal motor nucleus.

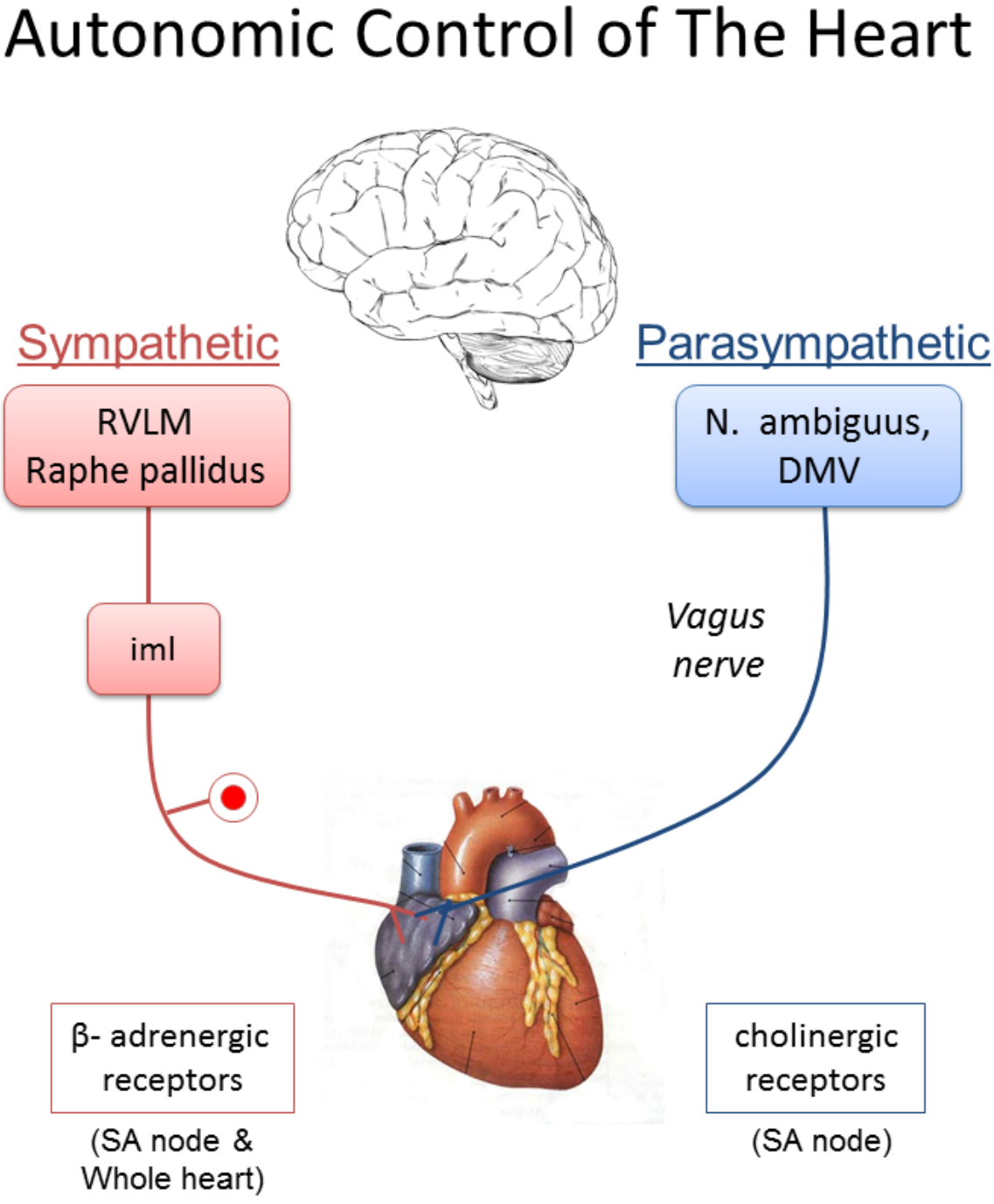


Figure 2: Schematic of sympathetic and parasympathetic control of the heart

1.3.2 Reduced heart rate variability is a marker of cardiac mortality risk

In a healthy cardiovascular system, the sympathetic and parasympathetic branches of the ANS work to ensure proper cardiovascular function under the varying conditions experienced by the organism. However, prolonged periods of chronic stress can have lasting effects on cardiac autonomic tone, characterized by an increase in sympathetic activity, a decrease in parasympathetic activity, or both. Time-domain HRV is a measurement of the variability in time between heart beats [i.e., the standard deviation of the inter-beat interval (IBI)], and reflects the interaction of sympathetic and parasympathetic influences on HR [22]. A reduction in HRV indicates a shift in sympathovagal balance toward higher sympathetic tone, and is a significant predictor of mortality following myocardial infarction [23, 24].

Depression is highly comorbid with a number of cardiovascular disturbances that fall under the general term of cardiovascular disease. Specifically, these include atherosclerosis, ventricular arrhythmia, myocardial infarction, and sudden cardiac death [7, 25-27]. Depression is also associated with increased risk of progression of heart failure [28]. Each of these cardiovascular pathologies, in turn, has been associated with prolonged sympathovagal imbalance, as measured by reduced HRV. Reduced HRV is consistently reported after myocardial infarction, predicts future cardiac pathology such as ventricular arrhythmia and sudden death [29], and is associated with higher incidence of cardiovascular mortality [30, 31]. Indeed, reduced HRV predicts the progression of atherosclerosis even more reliably than common risk factors such as smoking status or lipid measurements [32]. Additionally, reduced HRV is frequently observed in medically well depressed patients and reductions in HRV are significantly worse in severely depressed patients [21, 33]. Indeed, major to severe depression

doubles the cardiac mortality risk, as predicted by reduced HRV, in patients who are otherwise medically healthy [1].

While HRV in the time domain (discussed above) is useful as an index of overall autonomic influence of HR, frequency domain analysis of HRV can be used as an index of relative sympathetic and parasympathetic components that contribute to HRV. Frequency domain analysis identifies and measures rhythmic fluctuations in HR, and examines power (e.g. variability) as a function of frequency. The frequency distributions produced by this method are separated into frequency bands that have been shown to correspond to specific components of the ANS (**Figure 3**). Total power across the frequency spectrum is related to overall autonomic balance [22]. The high frequency (HF) band corresponds to the respiratory frequency, and is used as an index of vagal or parasympathetic influence on HRV [22, 34]. Low frequency (LF) HRV is influenced by sympathetic and parasympathetic elements [22, 35] and very low frequency (VLF) power likely reflects modulation by the renin-angiotensin system [29, 35, 36] or its effects on parasympathetic influence on the heart [36]. Reduced VLF is an especially useful measure, as it strongly predicts cardiac mortality, and accounts for up to 30% of the mortality risk of depression after myocardial infarction [31].

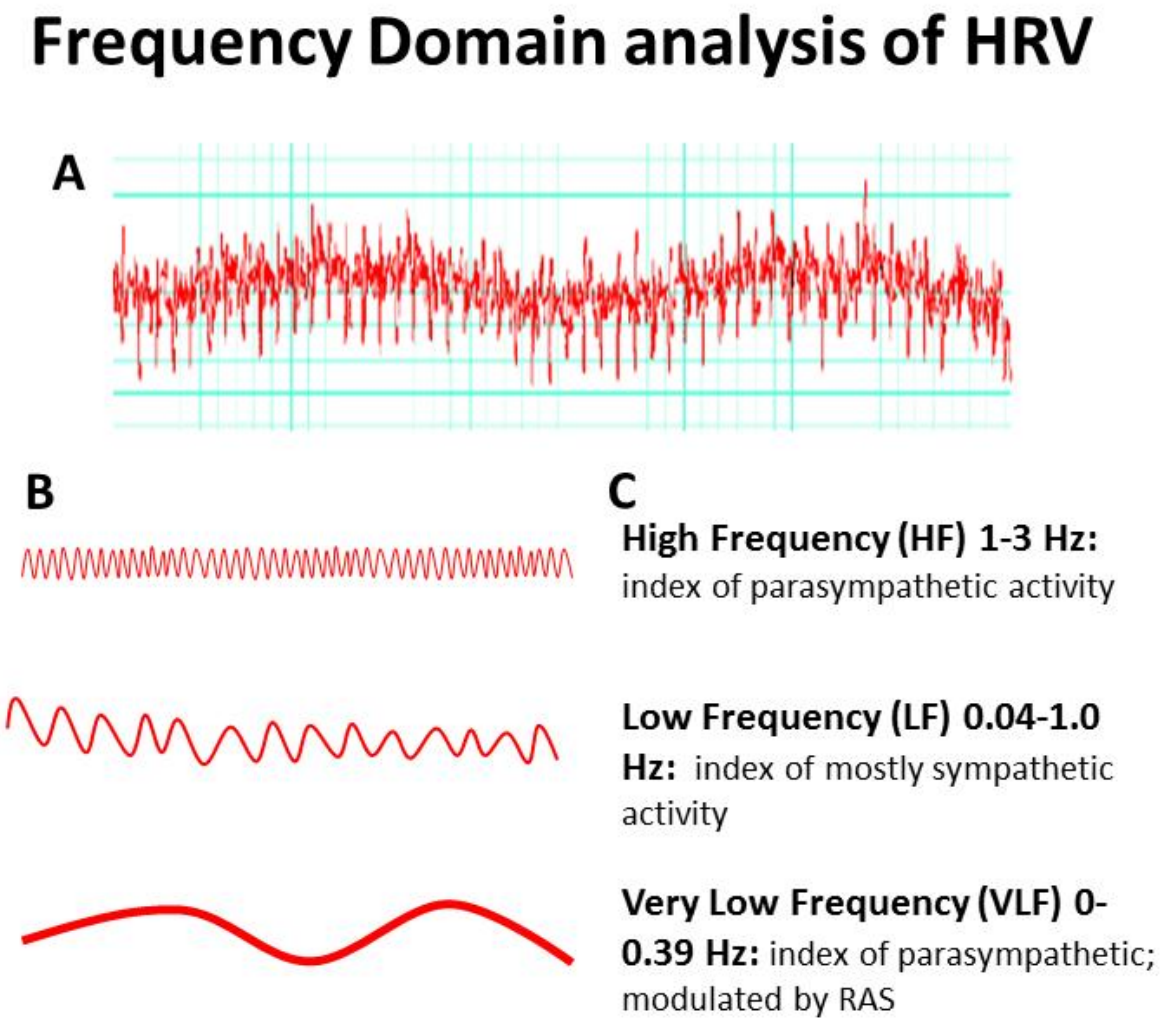


Figure 3: Diagram demonstrating the concept of frequency domain analysis.

Frequency domain analysis first separates waveform data (**Fig 3A**) into its component frequencies (**Fig 3B**) and then identifies and quantifies the amount of fluctuation (i.e. variability) in HR that is accounted for by a given frequency band. Pharmacological studies have shown that these frequency bands can be used as an index of autonomic function (**Fig 3C**).

1.4 ANIMAL MODELS OF DEPRESSION: UNPREDICTABLE CHRONIC MILD STRESS

The comorbid relationship between depression and cardiovascular disease has been identified and firmly established because of important work in clinical and epidemiological research [2-4, 6, 10, 20, 37-45]. However, work in experimental animal models has significantly contributed to a more complete understanding of the mechanisms that are common to both disorders. Of the available animal models of depression, one of the currently best-validated is the chronic mild stress (CMS) model of depression used in rodents [46, 47]. In this model, an animal is exposed to a variety of mild, yet unpredictable, stressors over the course of many weeks. CMS induces a constellation of altered behavioral, physiological, and neuroendocrine responses that closely resembles aspects of human depression [46]. The unpredictable nature of CMS is critical, and predictable stress has been shown to have an opposite effect, improving measures of mood and cognitive function in rats [48]. Depression-like behaviors have been evaluated by measuring anhedonia, one of the core signs of depression [12]. Anhedonia has been operationally defined using a variety of measures, including decreases in sucrose preference [49-53], approach to palatable food [54], brain stimulation reward [47, 55], and even palatable food-induced dopamine release in nucleus accumbens and prefrontal cortex [56, 57]. CMS-induced anhedonia is reversed by chronic, but not acute, administration of common antidepressants [46, 47, 53, 58-63], demonstrating that CMS is an appropriate model for studying the effects of chronic antidepressant treatment. CMS has proven to be a very useful tool for studying physiological and neural alterations that may take place during the development of depression [58, 64-66]. For instance, a series of studies that used CMS to induce anhedonic behavior in rats also reported changes in cardiovascular function that parallel those observed in depressed patients, including a

small increase in HR, decreased HRV, and an increased susceptibility to arrhythmia [49, 53, 67, 68]. However, a common criticism of the CMS model is that results can vary across experiments, even within labs. Indeed, in preliminary experiments performed in our lab, CMS-exposed rats had a modest but significant decrease in sucrose preference, which was variable across individual rats. Furthermore, preference for sucrose was positively correlated with pre-CMS locomotor activity in an open field. These results suggested that individual differences in behavioral traits might predispose certain rats to be vulnerable or resilient to the anhedonic effects of CMS.

1.4.1 Modeling vulnerability to depression: High- and low-responder rats

Although factors such as major life stress certainly contribute to the onset of depression, some individuals are more likely than others to become depressed. Indeed, depression is most likely aused by a complex interaction of genetic predisposition and environmental factors, and epidemiological studies estimate that genetic factors account for approximately 40-50% of risk for depression [11, 69]. Most individuals experience stressful life events, yet only a portion of individuals will experience serious anxiety or depressive disorders as a result of these events [70].

1.4.2 Novelty-seeking predicts emotionality in screened supplier-bred rats

One way to approach the issue of vulnerability and resilience has been to correlate baseline individual differences in behavior with either vulnerability or resistance to markers of mood disorders (e.g. behavior, genes) [71-75]. For instance, based on locomotor activity in a novel

environment, Sprague-Dawley rats can be classified as either high-responders (HiR), which have high levels of exploration, or low-responders (LoR) which have low levels of exploration [71, 74, 76-78]. Since low exploratory activity is interpreted as a sign of anxiety-like behavior due to rodents' innate aversion to novel, open spaces, it is not surprising that this classification predicts emotional reactivity in other behavioral tests. Rats classified as LoR have higher anxiety-like behavior than HiR rats in tests such as the elevated plus maze (EPMZ) and the light-dark box [71, 76, 79, 80].

1.4.3 Selectively-bred rats represent the extreme ends of a spectrum of behavior and mood

One approach that has been used to study trait differences in rats is to selectively breed animals at the opposite end of the normal distribution, and this strategy has recently been applied to locomotor activity in a novel environment. These selectively-bred strains (bred HiR [bHR] and bred LoR [bLR], respectively) are thought to represent the extremes of the normal distribution of the Sprague-Dawley population. Individual differences in anxiety-like behavior persisted and intensified as rats were selectively-bred on the trait of LoR or HiR [71, 81], making them a convincing model of inherited susceptibility or resilience to mood disorders. Indeed, as the results in Chapter 2 demonstrate, bHR and bLR rats appear to be resilient and susceptible, respectively, to CMS-induced depression-like symptoms, and CMS-exposed bLR are an especially robust model of anhedonic behavior.

1.5 ANTIDEPRESSANT TREATMENT: HOW CAN YOU MEND A BROKEN HEART?

If depression and cardiovascular disease are connected, does treating depression also improve decreased HRV? In the case of tricyclic antidepressants, the answer is decidedly no, as those drugs are known to have cardiotoxic properties [82-84], leading doctors to discourage their use in patients that are especially vulnerable to cardiovascular dysfunction [85, 86]. In the case of selective serotonin reuptake inhibitors (SSRIs), reports are much less clear. Some studies have reported that SSRIs improve cardiovascular outcomes and reduce mortality [19, 87], while others have found that SSRIs do not improve mortality risk, even when depression is in remission [88]. Finally, some studies have reported that SSRIs may actually exacerbate cardiac mortality, especially when administered in conjunction with beta-blockers, which are commonly prescribed for various cardiovascular disorders [89]. Cognitive behavioral therapy also improves signs of depression but does not alter cardiac mortality [90]. These varied results present the troubling possibility that in some individuals the cardiac mortality risk associated with depression may persist even when depression is treated.

1.6 THE RENIN-ANGIOTENSIN SYSTEM IN STRESS AND DEPRESSION

Many hormonal and neurohumoral systems are activated in both depression and heart disease. One of these systems is the renin angiotensin system (RAS), which has an established role in cardiovascular regulation [91, 92]. Heightened levels of angiotensin II, the peptide that mediates the effects of the RAS, are considered a risk factor for heart failure [93], and can directly damage

cardiac myocytes [94]. Furthermore, drugs that inhibit the RAS such as angiotensin II type 1 receptor (AT1R) blockers (ARBs) and angiotensin converting enzyme (ACE) inhibitors have been shown to reduce mortality from heart failure [95], possibly by reversing the reduction in VLF mentioned above [29, 35, 36].

In rats, the RAS is also known to modulate both the HPA axis and the sympathetic nervous system responses to stress. Angiotensin II increases sympathetic nerve activity, stimulates norepinephrine release, and directly activates sympathetic ganglionic cells [91, 96, 97]. AT1Rs are located at every level of the HPA axis [98, 99], and blockade with a centrally-acting ARB attenuates the HPA response to acute stress [100]. Chronic pretreatment with the ARB candesartan also greatly reduces anxiety-like behavior in rats [101], and prevents gastric ulceration associated with a chronic stress [102, 103].

There is also some evidence that drugs that inhibit the RAS may have antidepressant properties. There is some evidence indicating that ACE inhibitors improve mood and cognitive function in hypertensive patients [104, 105], and antidepressant use is lower in hypertensive patients taking ARBs or ACE inhibitors [106]. Finally, drugs that inhibit the RAS have positive antidepressant-like effects in the forced swim test in mice [107, 108], a behavioral test with high predictive validity for antidepressant drug efficacy.

1.7 COULD AN ANTAGONIST OF THE RENIN-ANGIOTENSIN SYSTEM BE USED TO TREAT COMORBID DEPRESSION AND CARDIOVASCULAR DISEASE?

Major depression and cardiovascular disease are highly comorbid, and depression is considered a significant risk factor for cardiac mortality. In fact, severity of depression is correlated with a

reduction in HRV, a clinical marker of risk for cardiac mortality [33]. Thus individuals who are more vulnerable to depression may be also be at risk for increased vulnerability to cardiovascular dysfunction. We hypothesized that bHR/bLR rats would (1) provide a model of increased resilience or susceptibility to depression-like behavior brought on by the CMS model of depression (Chapter 2) and (2) susceptibility to depression-like behavior would be associated with significant changes in cardiovascular function, such as decreased HRV, particularly in the VLF range (Chapter 3). Furthermore, typically prescribed antidepressants might not improve cardiovascular risk, though they reverse depressive behavior. We tested whether an antagonist of the RAS, a system known to be involved in both cardiovascular dysfunction and mood, would reverse both anhedonic behavior and changes in cardiovascular function induced by the CMS model of depression (Chapter 4). These studies demonstrate that bLR rats exposed to CMS provide a robust model for studying the relationship between depression and cardiovascular disease and present evidence that antagonists of the RAS alleviate both anhedonic behavior and cardiovascular changes induced by CMS.

2.0 NOVELTY-SEEKING BEHAVIOR PREDICTS VULNERABILITY IN A RODENT MODEL OF DEPRESSION

This chapter was published as: Stedenfeld, K. A., Clinton, S. M., Kerman, I. A., Akil, H., Watson, S. J., Sved, A. F. Novelty-seeking behavior predicts vulnerability in a rodent model of depression. Physiol Behav. 2011,103:210-6.

2.1 ABSTRACT

The onset of major depressive disorder is likely precipitated by a combination of heredity and life stress. The present study tested the hypothesis that rats selectivity bred on a trait related to emotional reactivity would show differential susceptibility or resilience to the development of depression-like signs in response to chronic mild variable intermittent stress (CMS). Male Sprague-Dawley rats that were bred based on the trait of either high or low locomotor activity in response to a novel environment were exposed to four weeks of CMS or control conditions. Changes in hedonic behavior were assessed using weekly sucrose preference tests and anxiety-like behavior was evaluated using the novelty-suppressed feeding test. During four weeks of CMS, bred low responder (bLR) rats became anhedonic at a faster rate and to a larger degree than bred high responder (bHR) rats, based on weekly sucrose preference tests. Measures of anxiety-like behavior in the novelty-suppressed feeding test were also significantly increased in

the CMS-exposed bLR rats, though no differences were observed between CMS-exposed bHR rats and their unstressed controls. These findings present further evidence that increased emotional reactivity is an important factor in stress susceptibility and the etiology of mood disorders, and that bHR and bLR rats provide a model of resistance or vulnerability to stress-induced depression. Furthermore, exposing bHR and bLR rats to CMS provides an excellent way to study the interaction of genetic and environmental factors in the development of depression-like behavior.

2.2 INTRODUCTION

Reliable animal models of depression are crucial to the success of preclinical studies of mood disorders such as major depressive disorder. Chronic mild stress (CMS), an animal model of depression first developed in the late 1980's, has gained favor in recent years for several reasons. First, it uses variable and unpredictable chronic intermittent stress, an established risk factor for depression [11, 109]. Second, it provokes changes in behavior and physiology that closely resemble the human disorder [46, 47]. Finally, CMS-induced depression-like signs can be reversed with antidepressant treatment [46, 60-62]. Additionally, the extended timecourse of the CMS paradigm, which typically lasts 4-6 weeks, is more appropriate for studying the effects of chronic drug treatment than acute measurements of so-called "despair behavior" such as the forced swim test [47, 110-113]. The depression-like behavior produced by CMS has typically been evaluated by measuring anhedonia, a key component of DSM-IV criteria for major depressive disorder. Reduced sucrose preference, for instance, can be reversed by chronic but not acute administration of anti-depressant drugs and is not changed by drugs that are ineffective as

antidepressants [58, 64, 65, 114]. The CMS model of depression in rodents strongly mimics both the symptoms of major depressive disorder and the time course of typical responsiveness to pharmacological treatment.

However, environmental factors are not solely responsible for the onset of depression, and a key question in the study of mood disorders is that of vulnerability and resilience among individuals [70]. Researchers have begun to address this issue by examining baseline individual differences in behavior that correlate with either vulnerability or resistance to behavioral, genetic, hormonal, and molecular markers of mood disorders [71-75]. One behavioral trait that has been examined in this regard in rats is locomotor behavior in novel environments [71, 74, 76-78]. Outbred Sprague-Dawley rats can be classified based on their activity level in a novel environment. Rats classified as high-responders (HiR) have high levels of exploratory activity, whereas rats with low activity in a novel environment are classified as low responders (LoR). Low exploratory activity is interpreted as a sign of anxiety-like behavior due to rodents' innate aversion to novel, open spaces. Likewise, increased exploration is considered indicative of less anxiety-like behavior. Since the HiR/LoR designation is based on exploration of a novel environment, it is not surprising that this classification predicts emotional reactivity in other behavioral tests. Specifically, outbred rats classified as LoR have higher anxiety-like behavior than HiR rats in the elevated plus maze, light-dark box, and the open field test [71, 76, 79]. LoR rats' tendency toward higher emotional reactivity is also apparent in the forced swim test, in which they display more depression-like behavior than HiR rats [80].

In order to study the possible interaction of genetics and behavioral phenotype in HiR versus LoR animals, rats have been selectively bred based on that trait [71, 81]. These selectively-bred strains of each type (bred HiR [bHR] and bred LoR [bLR], respectively)

represent the extremes of the normal distribution of the Sprague-Dawley population. The patterns of anxiety- and depression-like behavior discussed above have been preserved and enriched across generations of bHR and bLR rats [71, 81], raising the possibility that the bHR/bLR rat strains may represent a model of inherited susceptibility or resilience to mood disorders. We therefore hypothesized that studying bHR/bLR rats in the CMS model of depression would reveal an interaction of genetic predisposition and environmental stress, providing a model of vulnerability or resilience to stress-induced depression.

2.3 EXPERIMENTAL PROCEDURES

Animals and Housing

Rats (male, Sprague-Dawley) used in this study were selectively-bred for locomotor traits according to procedures outlined in Stead et al., 2006 [71] and Clinton et al., 2007 [81]. These rats were from the F20 generation bred at the Molecular and Behavioral Neuroscience Institute at the University of Michigan; 16 Low-Responder (bLR) rats and 15 High Responder (bHR) rats were shipped to the University of Pittsburgh when they were approximately 3 months old (400-450g). Prior to shipping, locomotor activity in a novel environment similar to the housing cage, was tested in all rats. All subsequent testing was performed at the University of Pittsburgh. Rats were housed individually in plastic cages (length x width x height: 40 x 22 x 19 cm) with 3-5 cm of bedding (course cut Aspen chips; P.J. Murphy) and wire lids. Standard rat chow (Purina) and tap water were available *ad libitum*. Rooms were kept at a constant temperature of 23°C under 12 h light: 12 h dark lighting conditions (lights on at 0700). CMS-exposed and Control groups

(see descriptions below) were housed in separate rooms under similar conditions. The University of Pittsburgh Animal Care and Use Committee approved all animal protocols that were used.

Chronic Mild Stress Protocol

After a two-week period to collect baseline measurements, rats were either exposed to CMS for a total of 5 weeks, or were handled according to standard animal care practices (Control group). CMS and Control groups were single-housed in separate rooms. The following individual stressors were used in the CMS protocol in varying order across different weeks (**Figure 4**): continuous overnight lighting; overnight water deprivation (18h) followed by 1 hour of empty water bottle replacement; 40-degree cage tilt; stroboscopic lighting (2-6 h; Chauvet mini-strobe CH-730; 8-12 flashes per second, 35 watts); overnight paired housing with another CMS-exposed rat (18h); damp bedding (300-500mL lukewarm water added to cage bedding); white noise (radio static, 85dB, 1-4 h, continuous or intermittent); and predator odor exposure (30-60 minutes exposure to 20uL undiluted 2,4,5-trimethylthiazoline (TMT; Pherotech Intl.) placed on a piece of filter paper and hung in each rat's cage). These mild environmental stressors were chosen to minimize the amount of interaction between experimenter and animal and have been shown in previous studies to be effective in inducing depression-like behaviors in rats [49-53, 58, 68, 111]. All CMS rats were exposed to the same schedule of stressors, although the schedule of these stressors was altered on a weekly basis and multiple stressors occasionally overlapped. However, overnight food- and water-deprivation was only combined prior to the sucrose preference test (below).

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Sucrose Preference Test						11a - 12p	
Food deprivation	6p-->	7:30a			5p-->	11a	
Water Deprivation			5p-->	9a	5p-->	11a	
Empty Water Bottle				9a-10a			
Overnight Illumination	7p-->	7a		7p-->	7a		
Cage Tilt		5p-->	9a			12p-4p	
Paired Housing				5p-->	9a		
Damp Bedding	5p-->	9a					
Intermittent White Noise			12p-4p				12p-3p
Strobe Light		2a-6a		1a-4a			
Predator Odor					12p-1p		

Figure 4: An example of a typical chronic mild stress (CMS) schedule.

CMS-exposed rats were exposed to a variety of intermittent stressors, which were applied each week, on different days and in different combinations. Control rats were housed in a separate room and were handled according to usual animal care protocols with the addition of a weekly sucrose preference test (SPT). SPT was always administered on Friday.

Sucrose Preference Test

The CMS protocol has been shown to decrease rodents' preference for sweet solutions (see [46] for a review), which is thought to represent anhedonia, a core symptom of major depression [12]. This anhedonic behavior is commonly assessed in rats via the sucrose preference test (SPT) [49-53, 64, 68, 114] and can be reversed by chronic antidepressant treatment [60-62]. To accustom the rats to the taste of sucrose, *ad libitum* water was replaced with 1% sucrose solution for one week. Tap water was returned one day before the first baseline SPT was administered. SPT was administered once per week to both CMS and Control groups. Rats were food- and water-deprived for 18 hours prior to the test. Two graduated burets were placed on the cage filled with either 1% sucrose solution or tap water. Rats were allowed to drink freely for one hour and total volume consumed was recorded. Preference for sucrose was calculated as [(mL sucrose / total mL consumed) *100].

Novelty-suppressed Feeding

The novelty-suppressed feeding test (NSF) is often used as a measure of depression-like behaviors. Like the open field test, the NSF test is based on rodents' innate fear of novel spaces. However, the NSF test introduces an additional component of motivation, as the food-deprived animal's drive to eat conflicts with its fear of novel open spaces. Chronic, but not acute, administration of antidepressants reduces these latencies, giving the NSF test excellent predictive validity for the timecourse of antidepressant efficacy [64, 115, 116].

During CMS week 5, rats were food-deprived 24 hours prior to the test, with free access to water and were moved to the dimly lit testing room one to two hours before the test. Rats were placed into one corner of an open field apparatus (17 in. x 17 in. x 12 in.) with clear acrylic walls

and an opaque white acrylic floor. Light level in the open field was maintained at 16-20 Lux and the walls and floor were wiped with Novalsan (chlorhexidine diacetate) between trials. A food pellet was placed in the center of the open field and rats were placed in one corner. Latencies to approach and to begin eating were recorded with a limit of 15 minutes. As soon as the rat was observed to eat, or the 15-minute time limit was reached, the rat was removed from the open field and placed in the home cage and observed until it began to eat in the home cage. Previous studies have demonstrated that home cage consumption is the same across treatment and control groups [64, 115, 116].

Data Analysis

Statistics were performed using Microsoft Excel and SPSS 16.0. Values are expressed as mean ± SEM. For comparisons of four groups, 2-way ANOVA (strain*treatment) was used, with Tukey HSD post-hoc tests where appropriate. Repeated measures ANOVA was used to identify significant differences in SPT measures across time, also with Tukey HSD post-hoc test. To compare proportion of animals in each group with SPT scores less than 50%, a χ^2-analysis was performed. Pearson correlation was used to examine the relationship between body weight and sucrose preference or fluid intake. For all tests, a two-tailed p-value of 0.05 or less was considered significant.

2.4 RESULTS

Body Weight

Body weights were measured once per week, at the same time that food and water were removed prior to the weekly sucrose preference test. At baseline, before exposure to CMS, there were no significant differences within strains (**Table 1**). However, body weights were significantly higher in bHR rats than bLR rats ($p<0.01$), a difference that persisted throughout the experiment and that we have observed in previous populations of bHR/bLR rats (unpublished observation, Clinton, Kerman, Akil & Watson). There was also a significant difference between treatment groups ($p<0.01$) in the total amount of weight gained during the experiment. While both strains of control rats gained similar amounts of weight, CMS-exposed rats did not gain weight during the experiment (**Table 1**).

Table 1: Comparison of body weight at baseline and at each of four weeks of CMS

	n	Baseline	CMS Week 1	CMS Week 2	CMS Week 3	CMS Week 4	Δ BW (g)
bHR - Control	7	553 ± 24*	570 ± 23*	581 ± 22*	589 ± 22*	576 ± 27*	22 ± 17
bHR - CMS	8	574 ± 21*	577 ± 23*	569 ± 22*	576 ± 22*	573 ± 20*	-1 ± 4**
bLR - Control	8	500 ± 10	505 ± 6	507 ± 6	515 ± 7	519 ± 7	19 ± 7
bLR - CMS	8	492 ± 19	495 ± 19	491 ± 19	489 ± 19	488 ± 19	-4 ± 7**

*: $p<0.01$ compared to opposite strain

**: $p<0.01$ compared to opposite treatment

Characterization of bHR/bLR rats

To confirm behavioral phenotype, bHR (n=15) and bLR (n=16) rats that were used in this study were exposed to a novel environment before being shipped to the University of Pittsburgh. As anticipated, bHR rats were markedly more active in a novel environment (**Figure 5**). The two groups were further assigned to CMS-exposed or control groups (n=8 per group, except bHR-Control n=7) and there were no baseline difference ($p > 0.05$) in locomotor scores between pre-assigned treatment groups (**Figure 5**). CMS-exposed rats were exposed to a variety of intermittent stressors, which were applied each week, on different days and in different combinations. Control rats were housed in a separate room and were handled according to usual animal care protocols with the addition of a weekly sucrose preference test (SPT). SPT was always administered on Friday.

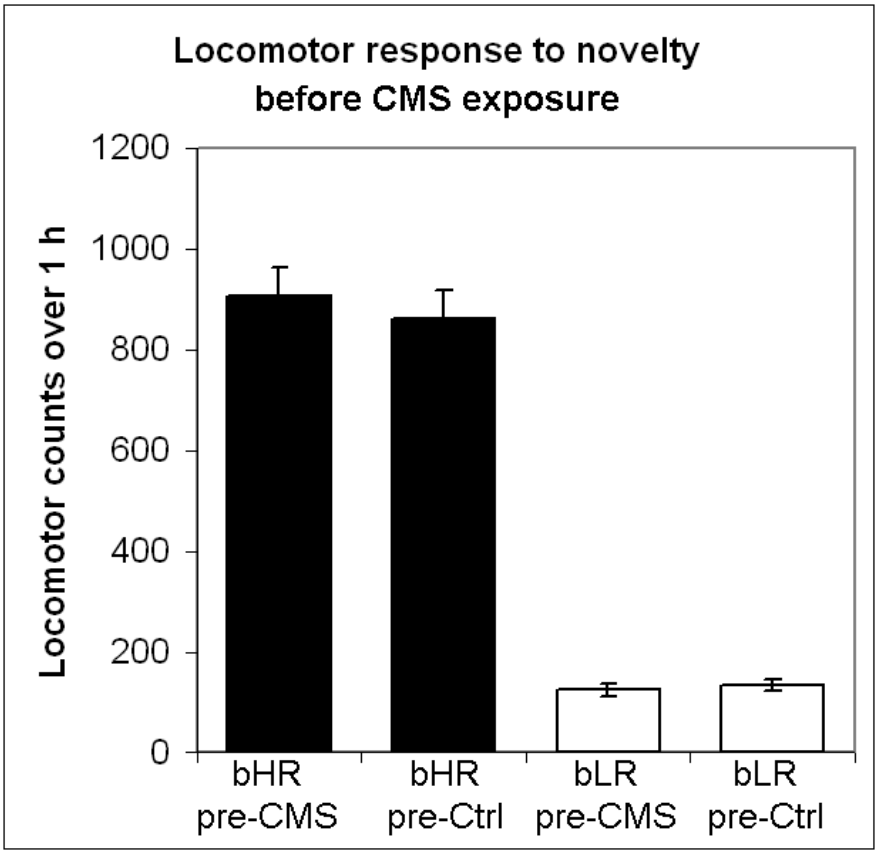


Figure 5: bHR and bLR pre-CMS locomotor activity in response to a novel environment

Prior to other experimental procedures, locomotor activity in response to a novel environment was tested. There was a significant difference between bHR (n=15) and bLR rats (n=16) in baseline locomotor activity ($p<0.01$) but no difference within strains in the subgroups of rats that were subsequently exposed to CMS or not.

Exposure to chronic mild stress

Rats of each rat strain were exposed to 4 weeks of intermittent CMS (**Figure 4**) and sucrose preference, used as a measure of anhedonia, was tested at weekly intervals. At baseline, all groups had similar robust preference for 1% sucrose versus water. In control rats of both strains, sucrose preference remained stable during the experimental period. In contrast, after two weeks of CMS, bLR-CMS rats showed significantly reduced sucrose preference ($p=0.01$; **Figure 6**) compared to both control groups and bHR-CMS rats ($p=0.02$), as well as their own baseline ($p<0.001$). bLR-CMS rats had SPT scores that were significantly less than 50% ($p<0.05$), which persisted throughout the CMS period. bHR-CMS rats did not have significantly reduced SPT scores until Week 4. Body weight was not correlated with either sucrose preference ($r = .023$, n.s.) or sucrose intake during the SPT ($r = 0.37$, n.s.).

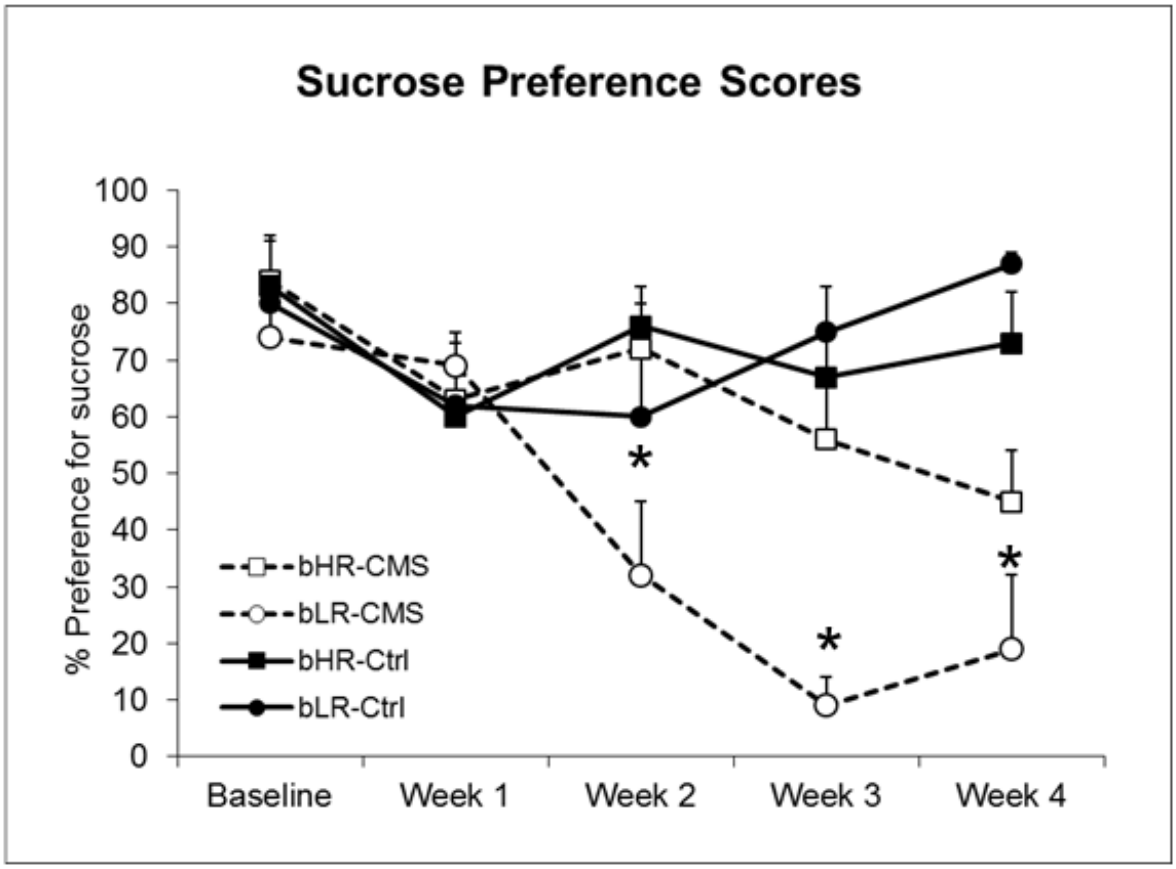


Figure 6: Percent preference for sucrose across four weeks of CMS

Sucrose preference was assessed at weekly intervals. At baseline and Week 1, groups had no significant difference in sucrose preference. bLR-CMS rats (n=8) had reduced sucrose preference compared to the other three groups starting in Week 2. In Weeks 3 and 4 there was a significant Treatment x Strain interaction ($p=0.02$). Values are means ± SEM. *:$p<0.05$ compared to other groups.

Performance in novelty-suppressed feeding test

The NSF test was used as an additional measurement of depressive behavior [64, 115-117]. Latency to approach the food and latency to consume the food were recorded, with longer latencies in each parameter considered indicative of anxiety-like behavior [64, 115-117]. There was a significant interaction of strain and treatment; the bLR-CMS group scored higher on both measures, taking significantly longer to approach (**Figure 7A**) and begin to eat the food pellet (**Figure 7B**). In fact, only one of the eight bLR-CMS rats ate the food pellet within the 15-minute time limit of the test. Scores were not significantly different between bHR-CMS rats and either control group (**Figure 7A & 7B**). All rats were observed eating chow within two minutes of being returned to the home cage, with no significant differences among groups. Finally, only bLR rats (both CMS-exposed and control) defecated during the NSF test, a factor that is often interpreted to indicate increased anxiety during behavioral tests [118-121].

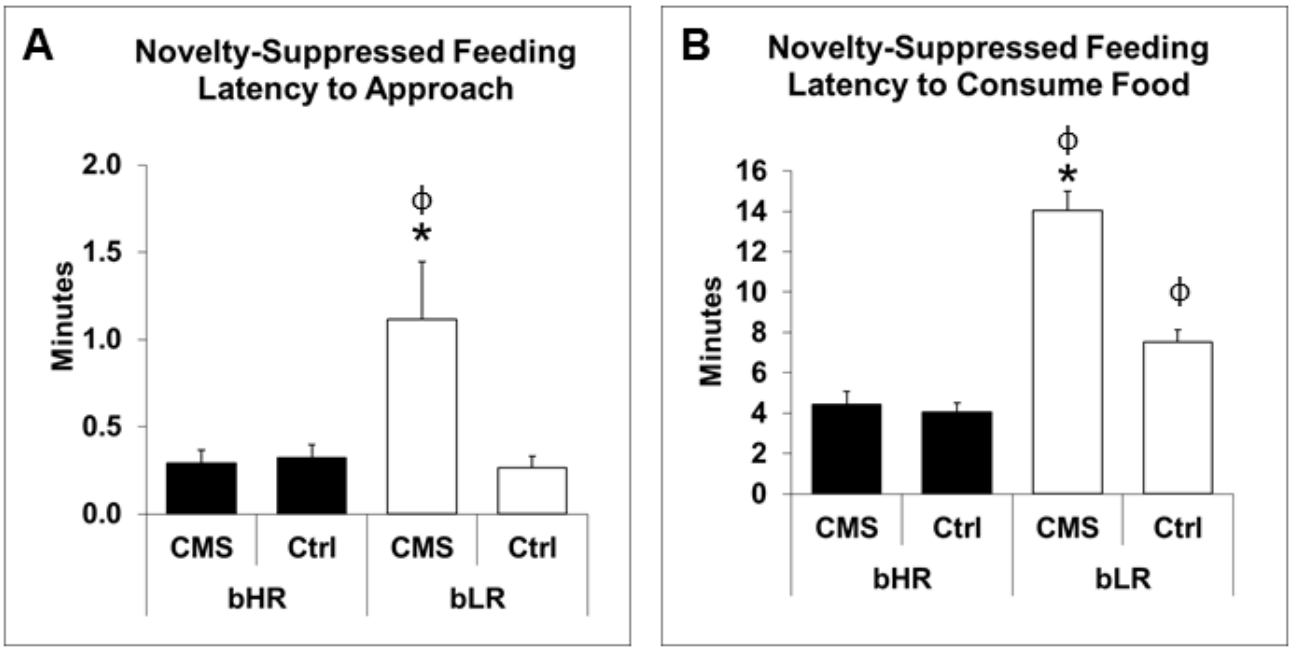


Figure 7: Latency to approach and consume a food pellet in the novelty-suppressed feeding test

Depression-related anxiety was further assessed in the Novelty-Suppressed Feeding test. Following 24 h food deprivation, bLR-CMS rats had increased latency to approach (4A) and eat (4B) a food pellet placed in the center of an open field. Values are means ± SEM. *: Significant difference from opposite treatment. ϕ: Significant difference from opposite strain. **7A**: Latency to approach food pellet. Significant effects of treatment ($p = 0.03$) and strain ($p = 0.05$) as well as an interaction of the two ($p = 0.03$) were found. **7B:** Latency to eat food pellet. Treatment, Strain, and Treatment*Strain: $p<0.001$

2.5 DISCUSSION

The key observation from these studies is that rats selectively bred for low and high locomotor responses to a novel environment show markedly different susceptibility to develop signs of depression in response to chronic mild intermittent stress. bLR rats showed signs of anhedonia sooner and to a greater degree than bHR rats, and this was confirmed with behavior in the NSF test, a test which is sensitive to antidepressant drugs [64, 117].

Supplier-bred Sprague-Dawley rats exhibit a range of behavior in novel environments [74, 122]. Based solely on this locomotor behavior, Sprague-Dawley rats can be separated into two groups: high-responders and low-responders (HiR & LoR), and these designations appear to predict emotional reactivity [71, 81]. The rats used in this study were selectively bred for novelty-seeking behavior in a novel environment over multiple generations. Differences in behavior and neurobiology have been well-characterized in bHR and bLR rats; in stressful or novel environments bHR rats have exaggerated locomotor activity, higher HPA activity and less anxiety [71, 81]. Additionally, bHR rats have increased drive for reward, are more inclined to self-administer drugs of abuse, and have higher dopaminergic tone in nucleus accumbens, compared to bLR rats [123, 124]. Based on these studies it has been postulated that differences in emotional reactivity between bHR and bLR rats represent a model of vulnerability to stress and mood disorders and may be a valuable tool for exploring genetic and environmental interactions of such disorders.

The current study used intermittent CMS as a model of stress-induced depression. CMS reproduces many of the complex symptoms typically observed in depressed human patients, including the core symptom of anhedonia, making it a highly useful animal model of depression [11, 46, 47, 60-62, 109-111, 113]. In rodents, anhedonia is most commonly measured

by a reduction in sucrose preference, which can be reversed by antidepressant drugs with a time course consistent with antidepressant efficacy and is not changed by drugs that are ineffective as antidepressants [58, 64, 65]. These results demonstrate the validity of the CMS time course in predicting antidepressant drug actions. After four weeks of CMS exposure, bLR rats showed significantly greater anhedonia, as compared to either bHR rats exposed to CMS or control (non-CMS-exposed) groups of both strains. It is notable that this reduction in sucrose preference in bLR rats occurred at a faster rate and to a greater degree compared not only to the bHR rats in this study, but also to supplier-bred Sprague-Dawley rats in studies from other labs [49-53, 68] including ours (Stedenfeld & Sved, unpublished observation). Furthermore, these scores were significantly less than 50%, the expected score if rats exhibited no preference for either sucrose or water. It is possible, then, that this markedly reduced preference may reflect not only a lack of interest, but an actual aversion to the previously-rewarding sucrose solution. Such extreme anhedonia is noted in severe cases of major depression in human patients, including the melancholic subtype [12, 125, 126], which may be more likely to occur following stressful life events and in patients with low sensation-seeking personalities [127-132]. The melancholic subtype also may be related to a reduction in reward processing [133, 134], paralleling similar observations in bLR rats such as a reduction in dopaminergic tone in nucleus accumbens [124] and decreased propensity to self-administer drugs of abuse [123]. Melancholic patients also display increased anxiety, increased HPA axis responses to stress [135, 136] and increased levels of stress-related neuropeptides in the paraventricular nucleus of the hypothalamus (PVN) [137].

Differences between bHR and bLR behavior were also seen in the NSF test, which is used to measure anxiety-like behavior as well as antidepressant efficacy [115, 116]. Although these may seem like two very different endpoints for a single test, there is, in fact a high rate of

comorbidity between anxiety disorders and depression [138, 139], and the two disorders share some overlapping characteristics such as irritability, sleep disturbances, and difficulty concentrating [12]. However, in addition to being a central symptom of anxiety disorders, anxiety itself is often a component of the negative affect associated with depression [140, 141]. Although anxiety is not explicitly stated in the diagnostic criteria for major depression [12], up to 90% of depressed patients report anxiety as a symptom [142]. Indeed, anxiety without depression is much more common than depression without anxiety [140, 141]. Depressed patients with anxiety symptoms at the time of remission are more likely to experience a relapse than patients without anxiety [143]. This is especially true in the melancholic subtype of depression discussed above [129, 130].

We found that bLR-CMS rats took a substantially longer time than bHR-CMS rats to approach and then to consume the offered food pellet. In fact, only one of the eight bLR-CMS rats ate within the fifteen-minute time limit of the test, though there were no significant differences in home cage consumption among groups. Home cage food consumption is an important control for this particular test and indicates that NSF behavior in the open field is due to increased anxiety in bLR-CMS rats, rather than a difference in appetite or food-related reward.

While feelings of anxiety may be a component of both depression and anxiety disorders, the core symptom of anhedonia is unique to depression, [12]. bLR rats show heightened anhedonia (reduced sucrose preference) as well as anxiety-like behavior in the NSF compared to bHR rats, suggesting that the bLR/bHR model is a model of susceptibility or resilience to depression, rather than anxiety disorder per se. These substantial contrasts in behavior are most likely due to the interaction of inborn changes in neurobiology and the stress of the CMS model.

There are a number of rat strains available that have been selectively bred for behavioral characteristics that may correspond to changes in mood or emotionality. One such strain is the Flinders Sensitive Line (FSL), which was originally derived by breeding Sprague Dawley rats for altered sensitivity to the acetylcholinesterase inhibitor diisopropyl fluorophosphate. This strain was later described as a putative model of depression because the rats exhibit behavioral and physiological similarities to depressed patients such as abnormalities in sleep and reduced appetite and psychomotor function [144, 145]. However, although the FSL rats share several characteristics in common with depression, they lack what many consider a key characteristic: anhedonia [144-147]. Interestingly, although both FSL and FRL rats develop signs of anhedonia in response to CMS [145, 147] they do not differ from each other in this regard. Thus, the FSL model lacks evidence of a key criterion of depression and the FSL/FRL rats cannot be considered a dichotomous model of vulnerability versus resistance to depression.

Another selectively-bred rat strain was developed by breeding Wistar rats based on either high (HAB) or low anxiety-like behavior (LAB) in the elevated plus maze (EPMZ) [148]. The heightened anxiety-like behavior in HAB rats extends to other similar behavioral measures of anxiety (e.g. light-dark box, [149]) and neurobiological correlates of increased anxiety, such as increases in corticotrophin releasing hormone (CRH) in the paraventricular hypothalamus of HAB rats, as compared to LAB rats [150]). It has been suggested that HAB rats may be a model of depression because of their increased immobility in the forced swim test (FST), a model of learned helplessness frequently used to screen for antidepressant drug efficacy[151]. However, the FST has proven to be more useful as a tool for drug discovery than as a standalone model of depression-like behavior, given that acute SSRI treatment increases active behaviors and decreases immobility in unstressed rats [151]. However, measures of anhedonia (e.g., SPT) do

not appear to have been reported for HAB rats at baseline and during CMS. Additionally, LAB rats show a marked increase in so-called antisocial behavior [149] and aggression [152, 153]. These findings suggest that the HAB/LAB model may be useful in the study of anxiety and social behavior, but, at present, it is difficult to compare HAB/LAB rats to bLR/bHR rats as a model of selective vulnerability to depression.

A different approach to developing animal models of neuropsychiatric disorders has been to target specific neurotransmitter systems. For example, a transgenic rat has recently been introduced that lacks the serotonin transporter (5-HTT) [154]. These rats, as well as mice lacking the 5-HTT, display evidence of increased behavioral anxiety and increased immobility in the FST [155-157]. The 5-HTT knockout rat also shows reduced 24-hour sucrose consumption [155]. These and other transgenic rodent lines with specific neurochemical alterations may be beneficial in determining the role of individual neurotransmitters in depression and have been suggested to be useful animal models for research on the mechanisms underlying depression and its treatment.

In summary, the present study demonstrates that bLR and bHR rats provide a model of vulnerability and resistance to depression-like behavior following a period of chronic intermittent stress. This model provides excellent opportunities to further study the interaction of genetic predisposition to mood disorders and environmental stress. Future studies will further elucidate the underlying neurobiology associated with these differences in behavior and will explore the relationship between depressive behavior and altered autonomic and cardiovascular regulation in bLR rats.

3.0 VULNERABILITY TO ANHEDONIC BEHAVIOR IS ASSOCIATED WITH CARDIOVASCULAR ALTERATIONS IN A RODENT MODEL OF DEPRESSION

3.1 ABSTRACT

Major depressive disorder and cardiovascular disease are highly comorbid. We hypothesized that rats with vulnerability to depression would also show susceptibility to depression-related changes in cardiovascular function. Male Sprague-Dawley rats that were selectively-bred based on either high or low locomotor response to a novel environment were exposed to four weeks of Chronic Mild Stress (CMS), a model of depression employing a series of unpredictable, intermittent, and variable mild stressors. We previously showed that bred low-responder (bLR) rats are more vulnerable to CMS-induced anhedonia compared to bred high-responder (bHR) rats, as measured by preference for a dilute sucrose solution. In the present study, radiotelemetry transmitters were implanted to chronically record heart rate (HR) and blood pressure (BP). Heart rate variability (HRV) and frequency domain analysis of HRV were calculated as clinically relevant measures of autonomic function. Before CMS began, BP and HR were similar in bLR and bHR rats except during the light period (7am-7pm) when BP was higher in bHR rats. During the fourth week of CMS, CMS-exposed bLR rats showed increased resting HR, decreased HRV, and decreased frequency domain measures related to parasympathetic function, in addition to increased cardiovascular reactivity to an acute mild stress. bLR rats have an

increased inherent sensitivity to CMS-induced depression-like behavior that is associated with vulnerability to significant cardiovascular alterations similar to those seen in patients with depression. This study further demonstrates the usefulness of the bLR/bHR model as a robust model of susceptibility and resistance to CMS-induced depression and associated cardiovascular and autonomic changes.

3.2 INTRODUCTION

Major depressive disorder is considered a significant risk factor for coronary heart disease and cardiac mortality [2, 3]. This comorbid relationship is independent of common risk factors such as increased body mass index, smoking, or preexisting cardiac pathophysiology [3]. Indeed, depressed but otherwise healthy patients with no history of cardiovascular disease are as likely to suffer an adverse cardiac event as patients with established cardiovascular disease [1, 2]. Furthermore, this association between depression and cardiovascular disease is bidirectional. For example, while approximately 5% of American adults suffer from depression, the prevalence of this disorder in patients who have survived a myocardial infarction is several-fold higher [5, 6]. This bidirectional relationship between depression and cardiovascular disease has been firmly established in the clinical and epidemiological literature [6, 10, 37].

The relationship between depression and cardiovascular disease has also received support from animal research. Much of this work has utilized one of the best-validated rodent models of depression, the depression-like state induced in rats by intermittent unpredictable chronic mild stress (CMS) [46, 47, 49-53, 67, 110]. CMS uses a series of mild, unpredictable changes in the rodents' environment to induce a depression-like syndrome. Perhaps the most crucial depression-

like sign produced by CMS is anhedonia, typically defined in rodents as a reduction in preference for sweet solutions [46, 47, 110, 158]. In addition to physiological changes such as increases in hypothalamic-pituitary-adrenal (HPA) axis activity and circulating pro-inflammatory factors [51, 52], CMS-induced anhedonia is associated with potentially detrimental alterations in cardiovascular function that mirror those observed in depressed patients, including increased resting heart rate (HR) and reduced HR variability (HRV) [50, 52, 53]. Reduced HRV is used clinically as an indicator of risk for cardiovascular disease and heart attack [3, 23, 31], and can be attributed to a shift in autonomic drive to the heart, typically reflecting an increase in sympathetic activation, a decrease in parasympathetic activation, or both, as reflected by spectral analysis of HR rhythm [34, 159].

In human patients, the severity of depression is related to the degree of cardiovascular disturbance. Reduced HRV and increased resting HR are significantly worse in severely depressed patients [21, 33]. Patients with major to severe depression who are otherwise medically healthy have twice the mortality risk as those with minor depression [1]. Furthermore, the risk of cardiac death in patients with coronary artery disease is higher in patients with moderate to severe depression and has been shown to persist and even increase in the years following hospitalization for coronary artery disease [38]. These findings suggest that individuals with increased vulnerability to depression or more severe forms of depression are at high risk of cardiovascular disease and cardiac death.

Recent data from our lab has shown that rats selectively-bred for low locomotor activity in a novel environment (bred Low Responder (bLR) rats) are especially vulnerable to depression-like behavior [160]. In rodents, low exploratory activity in a novel environment is interpreted as a sign of anxiety-like behavior, because rats have an innate aversion to novel, open

spaces. Conversely, increased exploration is considered indicative of less anxiety-like behavior. bLR rats and their bred-high-responder (bHR) counterparts have been selectively-bred based on this behavior, a distinction that has also been observed in behavior across a variety of tests of mood and emotionality [71, 76, 161]). For example, in behavioral tests such as the elevated plus maze and light-dark box, bHR rats display significantly less anxiety-like behavior than bLR rats [71]. Following exposure to CMS bLR rats show signs of anhedonia, demonstrated by a decreased preference for a dilute sucrose solution, more rapidly and to a much greater degree than bHR rats. This apparent vulnerability to CMS in bLR rats and resistance in bHR rats was also seen in anxiety-like responses in a novelty-suppressed feeding test [160].

The current study expands upon these behavioral findings, using cardiovascular data recorded from the same group of bHR and bLR rats to examine the comorbid relationship between depression and cardiovascular disturbances. Specifically, we hypothesized that bLR rats exposed to CMS would be especially susceptible to the cardiovascular changes that co-occur with depression-like behaviors, and that these changes would be more severe in bLR rats than bHR rats. This experiment also permitted an examination of potential differences in baseline cardiovascular function and responses to mild stress in bLR and bHR rats.

3.3 EXPERIMENTAL PROCEDURES

Animals and Housing

The present study represents an additional analysis of the bLR and bHR rats for which the behavioral consequences of CMS have already been reported [160]. Details of the behavioral studies and methods can be found in that report. As noted in that previous report, the bLR

(n=16) and bHR (n=15) rats used in these studies were from the F20 generation bred from Sprague-Dawley rats at the Molecular and Behavioral Neuroscience Institute at the University of Michigan and shipped to the University of Pittsburgh when they were approximately 3 months old (400-450g). At the University of Pittsburgh the rats were housed singly in plastic tubs under controlled environmental conditions with a 12/12 light dark cycle (lights on at 7 am). Food (Purina Chow) and tap water were available ad libitum except as noted as a part of CMS and testing procedures. All animal protocols conform with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh Animal Care and Use Committee.

Telemetry transmitter implantation

After one week to acclimate to the housing facility at the University of Pittsburgh, telemetry transmitters (PA-C40, Data Sciences International) were implanted in all rats to record BP, HR, and HRV. Under isofluorane anesthesia (2% in 100% oxygen, delivered at 1-1.5 L/min), the body of the transmitter was anchored to the abdominal muscle in the intraperitoneal space and the catheter was implanted into the femoral artery. Wounds were closed with silk suture and Ketoprofen (2mg/kg, s.c.) was administered as an analgesic. Rats were allowed 7-10 days to recover before baseline recordings began.

Chronic Mild Stress Protocol

After a two-week period to collect baseline measurements, rats were either exposed to CMS for a total of 5 weeks, or were handled according to standard animal care practices (Control group).

An example of the CMS schedule is shown in **Figure 4** (p.19) and details of the CMS protocol are described in our previous publication [160].

Sucrose Preference Test

Anhedonic behavior was assessed in rats at weekly intervals using a sucrose preference test (SPT). The testing methods and results have been reported in detail in Stedenfeld et al. [160] (Chapter 2).

Radiotelemetry Data Collection & Analysis

BP and HR measurements were collected using Dataquest ART 4.0 software (Data Sciences International) as a 10-second average every 2 minutes for the majority of the experiment. Continuous, beat-by-beat recordings of pulsatile BP were also made at selected points in the study in order to calculate HRV and components of its power spectrum. Due to hardware limitations, continuous recordings for HRV were taken from only 8 rats at a time, of the same treatment group (ie, CMS or Control), with 4 rats from each strain. HRV values are from the same time of day, but different days during the same week of the experiment. CMS was continued during recordings; therefore, recordings from CMS-exposed rats were sometimes taken during exposure to various ongoing stressors, including exposure to strobe light or white noise, water deprivation, and paired housing.

The telemetry transmitter also recorded activity level on the same schedule as BP and HR. The PA-C40 telemetry transmitter model records activity based on changes in transmitter signal strength, providing a crude index of overall activity.

HRV analysis was focused on the dark period, when rats are typically more active. Values from the first quarter of the dark period (time: 7pm – 10pm), when rats had a large increase in activity (data not shown) were evaluated, as were values from the third quarter of the dark period (1am – 3am). Time-domain HRV was calculated from continuous recordings of pulsatile BP, as the standard deviation of inter-beat-intervals (IBIs) within each of these two three-hour segments.

To assess autonomic contributions to HRV, frequency components of HRV were analyzed using the custom open source HRV analysis program, *Physioscripts*, which employs a band limited variance technique [34]. The sequence of IBIs was resampled at 10 Hz to create a regularly spaced time series. In addition to applying thresholds and limits to eliminate artifacts, sequences were also visually inspected for artifact, and missing or excluded points were replaced by linear interpolation. A bandpass filter was applied across the frequency band of interest before calculating the variance of the filtered time series. Boundaries for the frequency bands were selected based on previous studies in rats [162, 163]: total power (TP) 0-5 Hz, high frequency (HF) 1-3 Hz, low frequency (LF) 0.04-1.0 Hz, and very low frequency (VLF) 0-0.39 Hz. Frequency distribution analysis using this technique produces results analogous to spectral analysis using Fourier transform [22], but provides more robust results due to the filtering technique used, which omits frequencies outside of the specified bands [159].

Cage switch stress

Cage switch stress was used to assess cardiovascular reactivity to an acute mild stressor. During week 5 of CMS, HR and BP were continuously recorded for 50 minutes during the weekly cage change, in which the rat was transferred from a cage with week-old soiled bedding to a clean

cage with fresh bedding. This occurred during the light cycle, between 10:00am and 12:00pm; a similar cage transfer occurred once per week to all animals during the 8 weeks they were housed at the University of Pittsburgh.

Data Analysis

Statistical analyses were performed using Microsoft Excel and PASW Statistics 18.0 (SPSS). Values are expressed as mean ± SEM. For comparisons of four groups, 2-way ANOVA (Strain x Treatment) was used, with Tukey HSD post-hoc tests where appropriate. Repeated measures ANOVA was used to identify significant differences across time, with Tukey HSD test as appropriate. For all tests, a two-tailed p-value of 0.05 or less was considered significant.

3.4 RESULTS

Sucrose Preference Test

As we reported previously [160], CMS-exposed and control groups of both strains showed a robust preference for sucrose during the baseline period before CMS was started. During week 2 of CMS, CMS-exposed bLR rats showed a significantly reduced sucrose preference (compared to their baseline control, as well as the bLR-Control group and both bHR groups). This persisted throughout the remainder of the experiment. In contrast, the bHR showed a mildly reduced sucrose preference only after 4 weeks of CMS.

Baseline cardiovascular measurements in bHR and bLR rats

At baseline, before CMS began, values for HR and BP during the light and dark period were within the typical range for adult Sprague-Dawley rats (**Figure 8A-D**) and showed a clear circadian pattern. Baseline HR values did not significantly differ between bLR and bHR rats during the light or dark period, though they tended to be higher in bHR rats. bHR rats had significantly higher resting BP than bLR rats during the dark period ($p<0.05$), but not the light period. Baseline HRV values were not significantly different. Activity, assessed by changes in telemetry signal strength as the rat moves about its cage, showed a strong circadian pattern, with increased activity during the dark phase. As expected and reported previously [71, 81, 160], bHR rats were more active across the 24-hour period than bLR rats ($p=0.003$, data not shown).

Heart rate and blood pressure during CMS exposure

HR was significantly elevated in bLR-CMS rats compared to bLR-Control and bHR-CMS rats during the dark phase of weeks 3 and 4 of CMS (**Figure 8A,** $p < 0.05$). There were no significant differences in HR during the light period during any week of CMS (**Figure 8B**). There were also no significant differences in BP between CMS-exposed and control groups at any time point (**Figure 8C & 8D**). However, bHR rats had significantly higher BP than bLR rats during the light period of CMS week 4 (**Figure 8D,** $p=0.003$); this effect was mainly driven by the significant difference between the bHR-Control group, and the bLR-Control group ($p=0.05$). Additionally, the difference between light-period and dark-period HR and BP values did not change significantly over the four weeks of CMS, suggesting that cardiovascular circadian rhythms remained intact. Circadian rhythms in activity were also intact for all groups and there

were no significant differences in 24-hour activity between CMS-exposed and Control rats of either strain (data not shown).

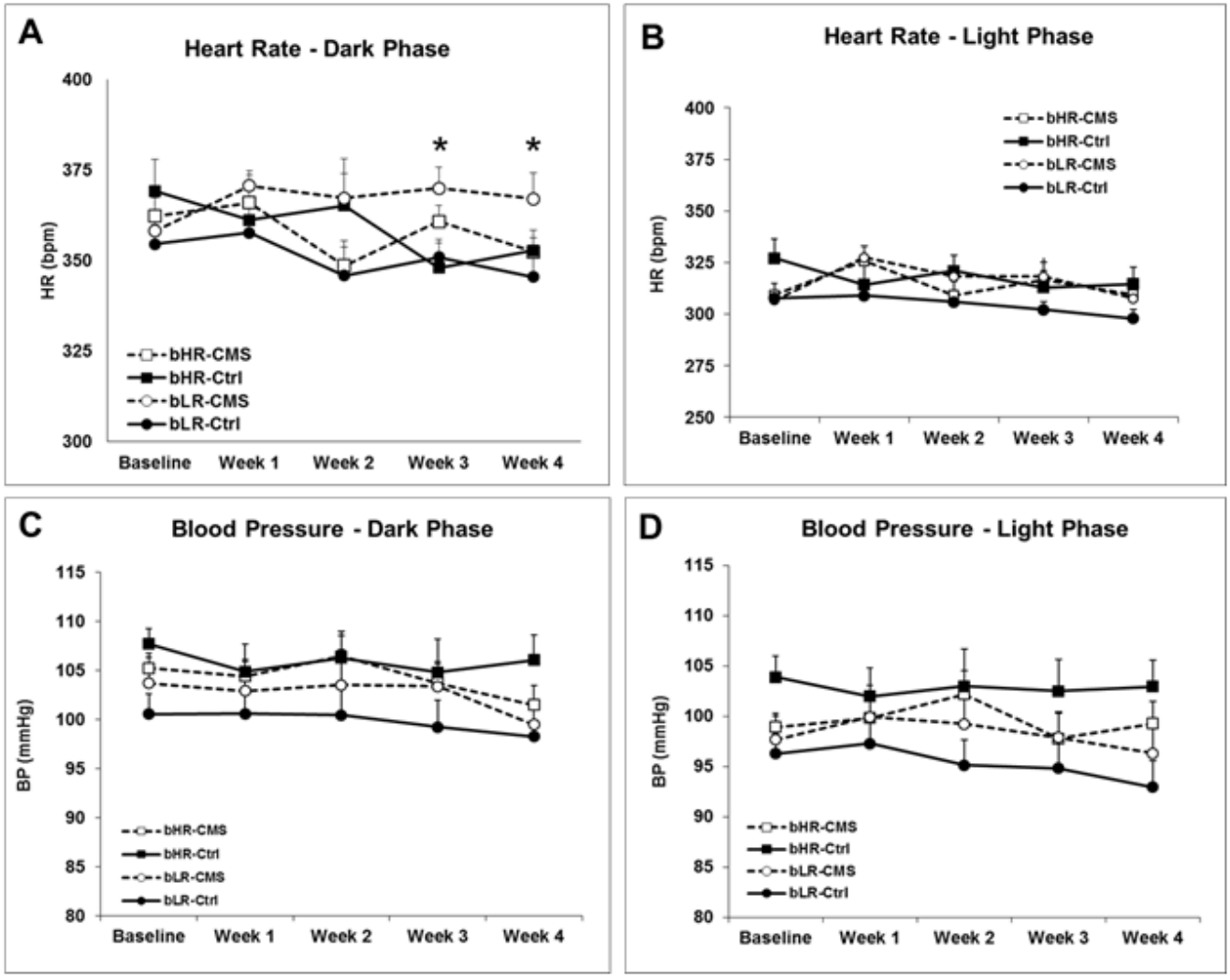


Figure 8: 24-hour resting heart rate and blood pressure

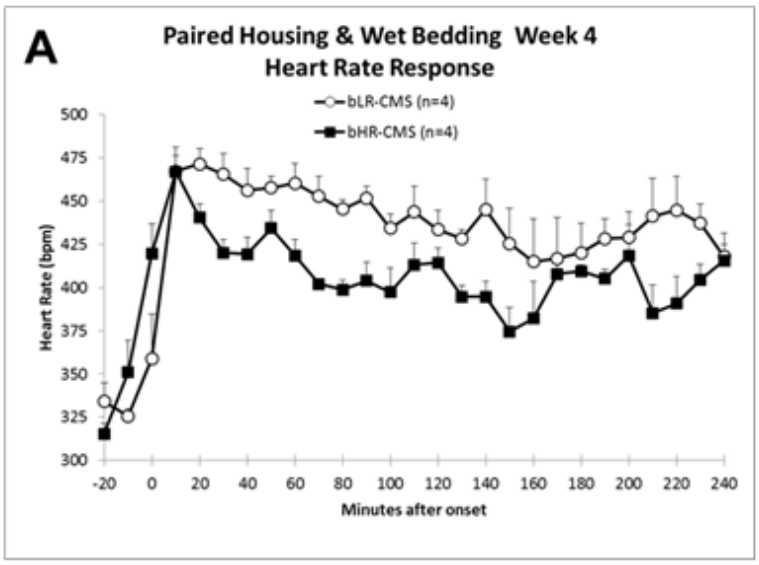
HR and BP were recorded chronically throughout the experiment, across the dark period (7pm-7am) and the light period (7am-7pm). HR during the dark phase was elevated in bLR-CMS rats during weeks 3 and 4 of CMS (**Fig 8A**). There were no significant differences in HR during the light phase (**Fig 8B**), BP during the dark phase (**Fig 8C**), or BP during the light phase (**Fig 8D**). Values are means ± SEM; n=8/group except bHR-Ctrl, n=7. *:$p<0.05$ compared to Controls.

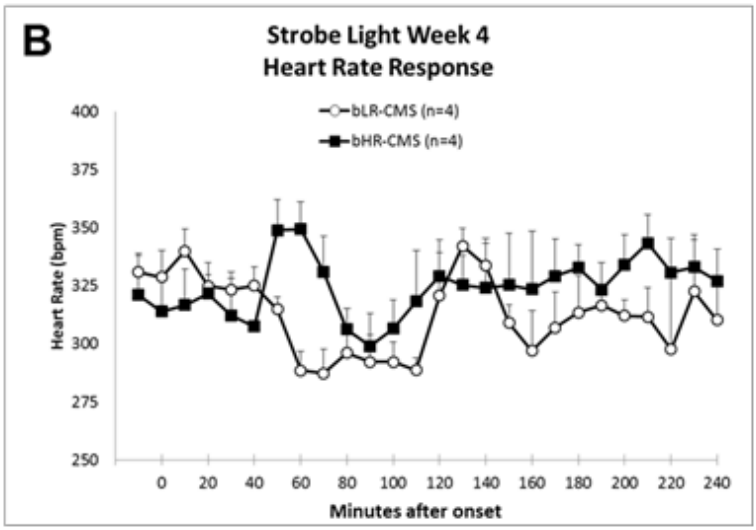
Cardiovascular response to CMS protocol stressors

BP and HR were recorded throughout the CMS protocol, allowing for analysis of the impact of the various stressors on these parameters. However, during continuous overnight recordings of beat-by-beat data for HRV analysis only 8 rats could be recorded at one time. Therefore, this data set was not entirely comprehensive, but includes cardiovascular data from smaller groups of rats during a subset of stressors. We have chosen to present data from a few of these stressors as representative examples of cardiovascular responses to a range of CMS stressors. The following stressors were chosen because recordings were available for both bLR-CMS and bHR-CMS rats for at least two of the four weeks of CMS: overnight wet bedding plus paired housing, strobe light, and loud white noise. The combination of overnight wet bedding and paired housing produced the largest and most sustained HR response (**Table 2, Figure 9A**). During this weekly stressor, CMS-exposed bHR (n=4) and bLR rats (n=4) were paired with another CMS-exposed rat of either the same or opposite strain. The same eight rats remained in their home cage and were recorded from each week and were always paired with an unfamiliar rat of similar body weight whose transmitter was turned off. Once the intruder rat was placed in the cage, 300-500mL of lukewarm water was poured on the bedding, often wetting the rats as well. Aggressive behavior was sometimes observed in the first 30-60 minutes of paired housing, but was never sustained or severe enough to require separation. Following onset of the stressor, all rats had a significant increase in HR and BP (**Figure 9A**). There were no significant differences in peak HR response between bHR and bLR rats in any of the 4 weeks of CMS **(Table 2)**. Peak response was defined as the difference between the highest recorded value in the 15 minutes following stressor onset and at t = -10 minutes. During each of the four weeks of CMS, bLR-CMS rats had a more sustained HR response than bHR-CMS rats, as measured by a larger area

under the curve (AUC), for the first 240 minutes (4 hours) of stress ($p<0.01$, **Table 2**). There were no significant differences between bLR-CMS and bHR-CMS rats in peak BP response or AUC during any of the 4 weeks of CMS.

Most stressors used in the CMS protocol, however, did not produce large cardiovascular responses. For example, a strobe light turned on during the dark period elicited a very small increase in HR and BP (**Figure 9B**). Peak HR response and AUC were not significantly different between bLR-CMS and bHR-CMS rats. Similarly, loud (85dB) white noise caused a small and transient increase in HR and BP (**Figure 9C**). The peak HR response to white noise was significantly larger and more sustained in bLR-CMS rats than bHR-CMS rats during CMS week 2 ($p<0.01$, **Table 2**). For both of these stressors, there were no significant differences in peak BP response or AUC for BP response between bLR-CMS and bHR-CMS rats. These data demonstrate that the CMS protocol produced small to moderate increases in HR and BP, and that these changes were sometimes larger or more sustained in bLR rats than bHR rats.





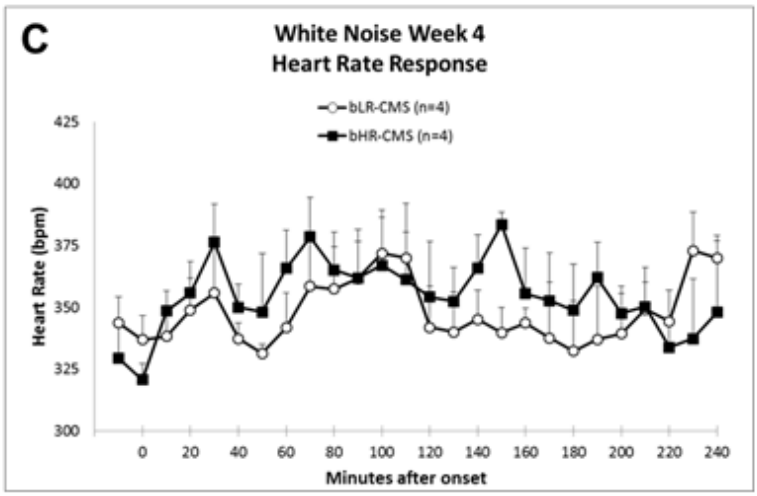


Figure 9: Heart rate responses to three CMS stressors during CMS week 4

9A: HR response to Paired housing plus wet bedding. CMS rats were paired overnight in a cage with dampened bedding. There was no significant difference in peak HR response, but bLR-CMS rats had a longer duration of HR response, measured by AUC ($p<0.01$).

9B: HR response to Strobe light during the dark period. There were no significant differences in peak response or duration.

9C: HR response to white noise. There were no significant differences in peak response or duration.

Table 2: Peak and duration of heart rate and blood pressure responses to three CMS stressors

		Peak Increase in HR (bpm)				Area Under the Curve - HR response			
		Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4
Paired Housing and Wet Bedding	bLR-CMS (n=4)	149±7	123±15	116±14	152±2	105292±1374#	98790±3889#	104261±2170#	105597±2292#
	bHR-CMS (n=4)	139±4	116±9	109±21	122±10	96634±1406	85036±743	93660±980	98144±710
Strobe Light	bLR-CMS (n=4)	NA	NA	13±7	19±20	NA	NA	77956±2578	73165±1174
	bHR-CMS (n=4)	NA	NA	11±11	10±3	NA	NA	77647±2061	77896±2057
White Noise	bLR-CMS (n=4)	NA	50±6#	NA	23±9	NA	66782±2033#	NA	86274±1150
	bHR-CMS (n=4)	NA	34±7	NA	31±8	NA	73695±1290	NA	84993±2839

		Peak Increase in BP (mmHg)				Area Under the Curve - BP response			
		Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4
Paired Housing and Wet Bedding	bLR-CMS (n=4)	18±5	7±3	19±2	15±1	26043±1590	25166±672	25978±940	26767±911
	bHR-CMS (n=4)	11±5	12±3	23±5	15±3	27186±261	24679±228	25836±323	28088±277
Strobe Light	bLR-CMS (n=4)	NA	NA	3±2	2±3	NA	NA	22855±602	21602±672
	bHR-CMS (n=4)	NA	NA	7±2	3±5	NA	NA	23098±730	23005±899
White Noise	bLR-CMS (n=4)	NA	1±0.5	NA	1±1	NA	23243±417	NA	23966±973
	bHR-CMS (n=4)	NA	-1±1	NA	-1±1	NA	23499±1143	NA	25817±562

Peak increase and area under the curve (AUC) were calculated for HR and BP responses to each of three CMS stressors: paired housing plus wet bedding, strobe light, and white noise. Repeated measures ANOVA yielded no significant effects for any of these measures. Values are means ± SEM. #$p<0.01$ compared to opposite strain (one-way ANOVA).

Heart Rate Variability

Time-domain HRV (s.d. of the IBI) was measured each week throughout the CMS period. Analysis of HRV focused on the dark period, when rats are more active, and for which we have lengthy continuous cardiovascular recordings. During the first quarter of the dark period, there was a significant overall effect of strain ($p=0.03$) and stress ($p=0.006$), as well as a significant interaction of time and stress ($p=0.05$) (**Figure 10**). Baseline (pre-CMS) values are not included in this analysis because they were not recorded across the dark period; however, as noted above, baseline HRV values were not significantly different among groups (data not shown). There were also no significant differences among groups during weeks 1-3 of CMS. During CMS week 4, bLR-CMS rats had significantly reduced HRV compared to bLR-Control ($p<0.01$) and bHR-CMS ($p<0.05$) groups (**Figure 10**). bHR-CMS rats also had reduced HRV compared to their controls ($p<0.01$).

Frequency domain analysis of HRV was performed to evaluate possible sources of the reduction in HRV. During the fourth week of CMS, both bLR-CMS and bHR-CMS rats had reduced total power compared to unstressed controls ($p<0.05$, **Figure 11A)**. bLR-CMS rats had a significant reduction in HF power (**Figure 11B**) compared to controls ($p<0.05$), and bLR rats had lower LF power than bHR rats ($p<0.05$), regardless of whether they were exposed to CMS or not (**Figure 11B**). Both bLR-CMS and bHR-CMS rats had reduced VLF power, compared to unstressed controls ($p<0.05$, **Figure 11D**).

Interestingly, this significant reduction in HRV in bLR-CMS and bHR-CMS rats was not evident across the entire dark period. During the third quarter of the dark period (0100 – 0400), for instance, there was not a significant difference in HRV during the fourth week of CMS

(**Table 3**). Furthermore, there were no significant differences in frequency domain components of HRV, with the exception of an increase in LF power in bHR-CMS rats ($p<0.01$, **Table 3**).

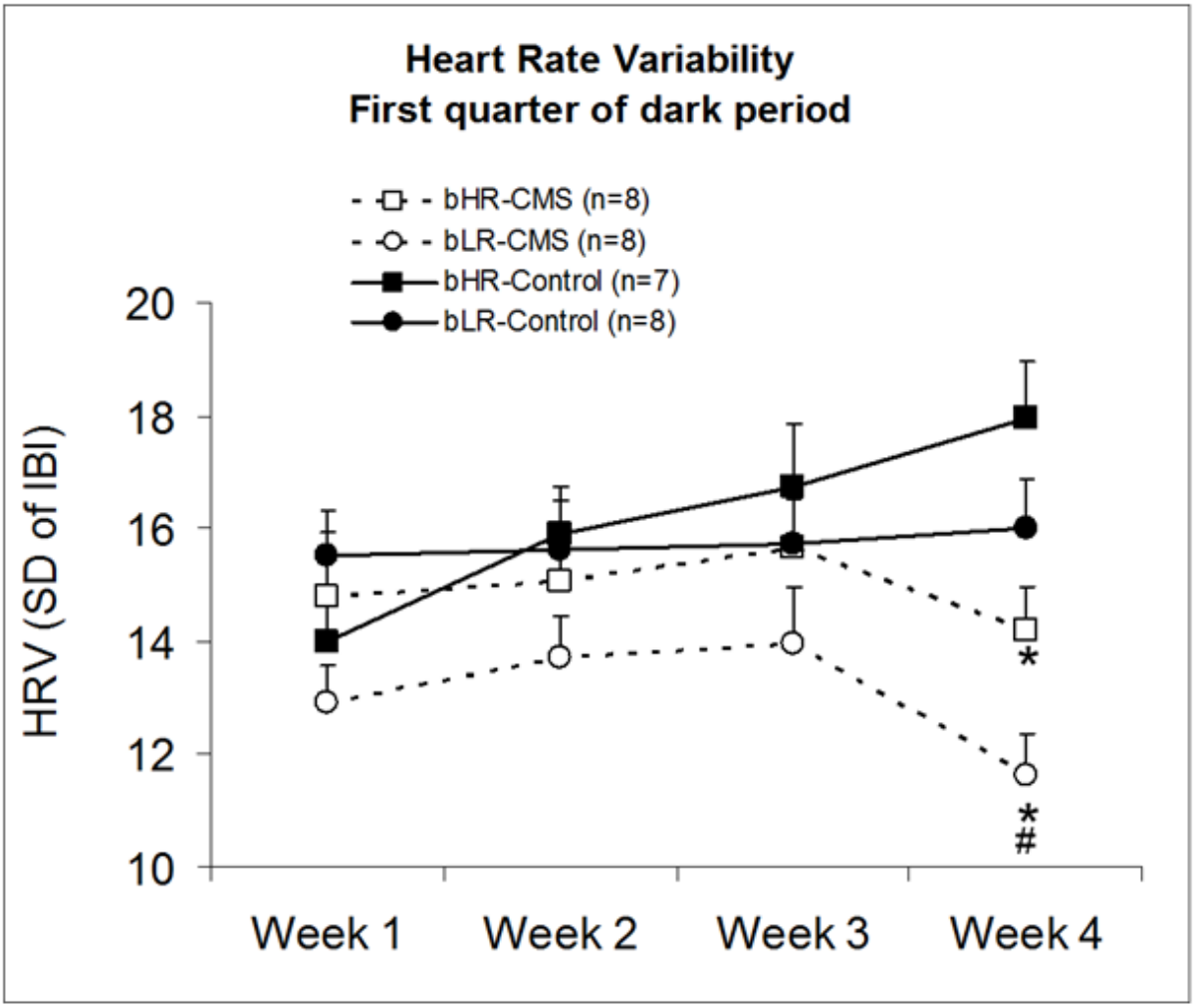


Figure 10: Time-domain HRV in bHR/bLR rats across four weeks of CMS

HRV was measured during the first quarter of the dark period (7pm-10pm). A repeated measures ANOVA yielded a significant effect of Strain ($p=0.03$), Stress ($p=0.006$) and a significant interaction of Stress x Time ($p=0.05$). HRV was significantly reduced in bLR-CMS rats compared to bLR-Control rats ($p<0.01$) and bHR-CMS rats ($p<0.05$). bHR-CMS rats also had reduced HRV during the fourth week of CMS. Values are means ± SEM. *:$p<0.01$ compared to Controls; #:$p<0.05$ compared to opposite strain.

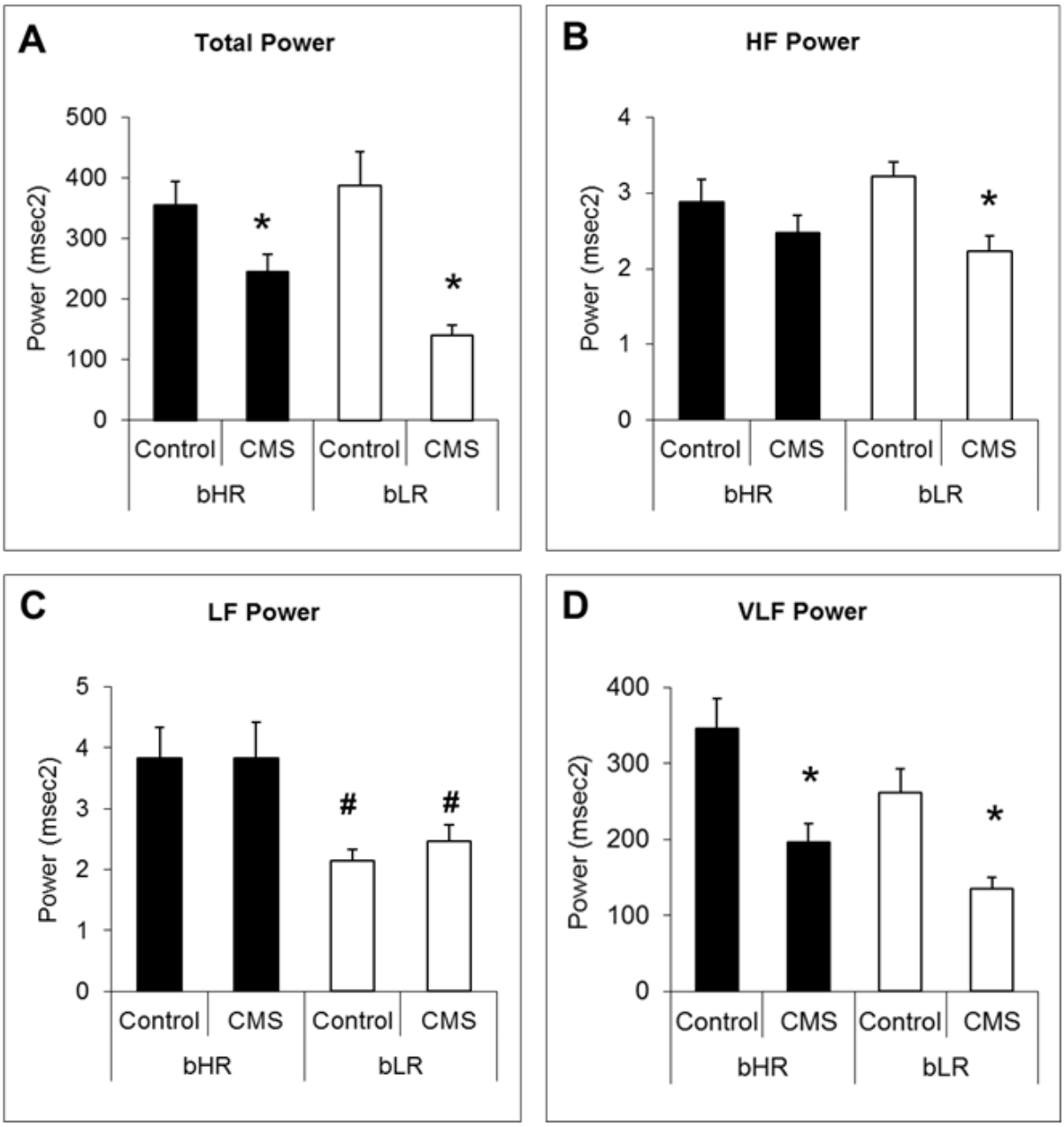


Figure 11: Frequency domain components of HRV, final quarter of the dark period

To assess autonomic contributions to HRV, frequency domain components were also calculated during the first quarter of the dark period. **11A:** Total Power (0-5Hz), **11B:** high frequency power (HF, 1-3Hz), **11C:** low frequency power (LF, 0.04-1.0 Hz), **11D:** very low frequency (VLF, 0-0.39 Hz). Values are means ± SEM. *:$p<0.05$ compared to Controls; #:$p<0.05$ compared to opposite strain.

Table 3: Frequency domain components of HRV, third quarter of the dark period

	HRV	HF	LF	VLF	TP
bHR-Control	14±1.2	3.0±0.3	2.2±0.5	213±31	220±31
bHR-CMS	15±0.8	3.3±0.2	4.0±0.2*#	220±22	229±23
bLR-Control	15±0.9	3.3±0.2	2.0±0.2	234±28	240±28
bLR-CMS	15±1.0	2.7±0.3	2.5±0.2	244±39	251±39

Values are means ± SEM. *:$p<0.01$ compared to Controls; #:$p<0.01$ compared to opposite strain.

Cardiovascular reactivity to an acute novel stress

During Week 5 of CMS, HR and BP were monitored during an acute stress to evaluate cardiovascular reactivity. Baseline BP and HR were recorded 10 minutes before being placed in a cage with clean bedding. All groups were within the normal range for Sprague-Dawley rats at rest. Just before the cage change there was a significant baseline effect of CMS for both HR and BP, which was mainly due to a significant difference in HR ($p=0.03$) and BP ($p=0.04$) between bHR-Control rats and bLR-CMS rats; there were no other significant differences among groups at baseline.

Immediately following placement in a cage with clean bedding, all groups showed a significant increase from baseline in both HR and BP (**Figures 12A & 12C**). Both strains of CMS-exposed rats had significantly higher peak increases in BP compared to controls ($p < 0.05$; **Figure 12D**). bLR-CMS rats had a significantly larger peak increase in HR compared to bHR-CMS rats ($p = 0.02$) and both control groups ($p = 0.004$; **Figure 12B**). There was also a significant main neffect of stress on the area under the curve (AUC) of cardiovascular responses during the first 40 minutes following stressor onset (i.e., t=0-40). AUC for HR (**Figure 13A**) and BP (**Figure 13B**) responses to novel cage placement was higher in bLR-CMS rats compared to bLR-Control rats (one-tailed ANOVA, $p = 0.02$ and $p = 0.03$, respectively). Additionally, there was a significant main effect of stress on the duration of the HR and BP responses (one-tailed ANOVA, $p = 0.05$ and $p = 0.04$, respectively). bLR-CMS rats had HR and BP responses that remained elevated above baseline for significantly longer than bLR-Control rats (**BP**: bLR-CMS: 41±7min vs. bLR-Control: 22±3min, $p=0.03$; **HR**: bLR-CMS: 49±7min vs. bLR-Control: 27±5min, $p=0.02$). This relationship was not observed in bHR rats (**BP**: bHR-CMS: 30±4 vs. bHR-Control 28±8, $p=0.40$; **HR**: bHR-CMS: 42±9 vs. bHR-Control 43±15, $p=0.48$).

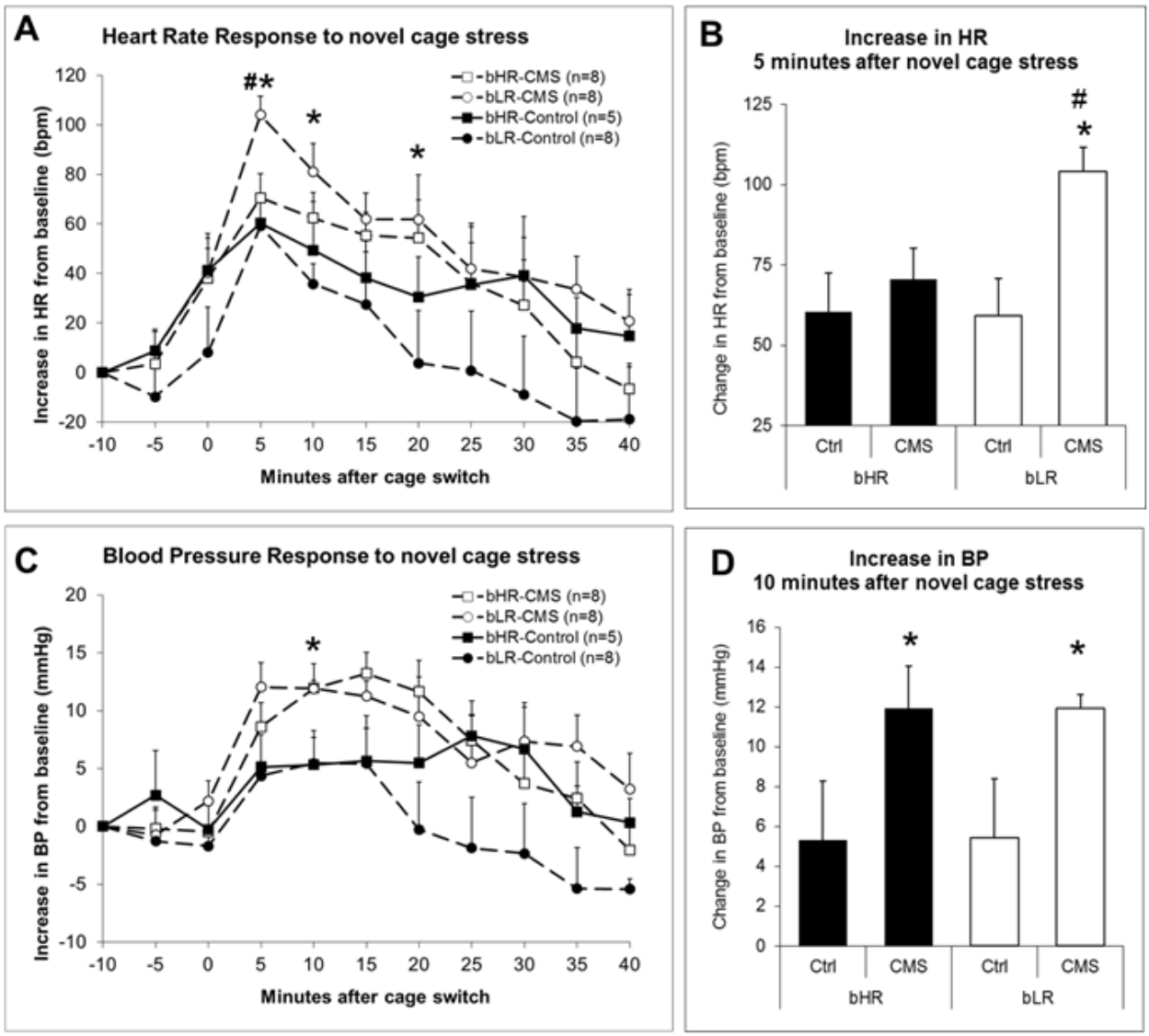


Figure 12: Increases in HR and BP in response to a novel cage stress in bHR/bLR rats

HR and BP increases from baseline after rats were transferred to a new cage with clean bedding. Values are means ± SEM. n=8/group, except bHR-Control, which had n=7 and two recordings from this group were not usable, thus n=5. *: $p<0.01$ compared to Controls; #: $p<0.01$ compared to opposite strain. **12A:** bLR-CMS rats had the largest peak increase in heart rate. Baseline heart rate (bpm); t = -10 minutes: bHR-Ctrl: 329±17, bHR-CMS: 291±12, bLR-Ctrl: 317±20, bLR-CMS: 268±7. **12B:** Bar graph of peak increase in heart rate from baseline 5 minutes after cage switch was performed. **12C:** Both strains of CMS-exposed rats had significantly higher peak blood pressure responses compared to controls. Baseline blood pressure (mmHg), t = -10 minutes: bHR-Ctrl: 104±4, bHR-CMS: 95±3, bLR-Ctrl: 99±4, bLR-CMS: 92±1 (significant effect of treatment, $p<0.05$). **12D:** Bar graph of peak increase in blood pressure from baseline 10 minutes after cage switch was performed.

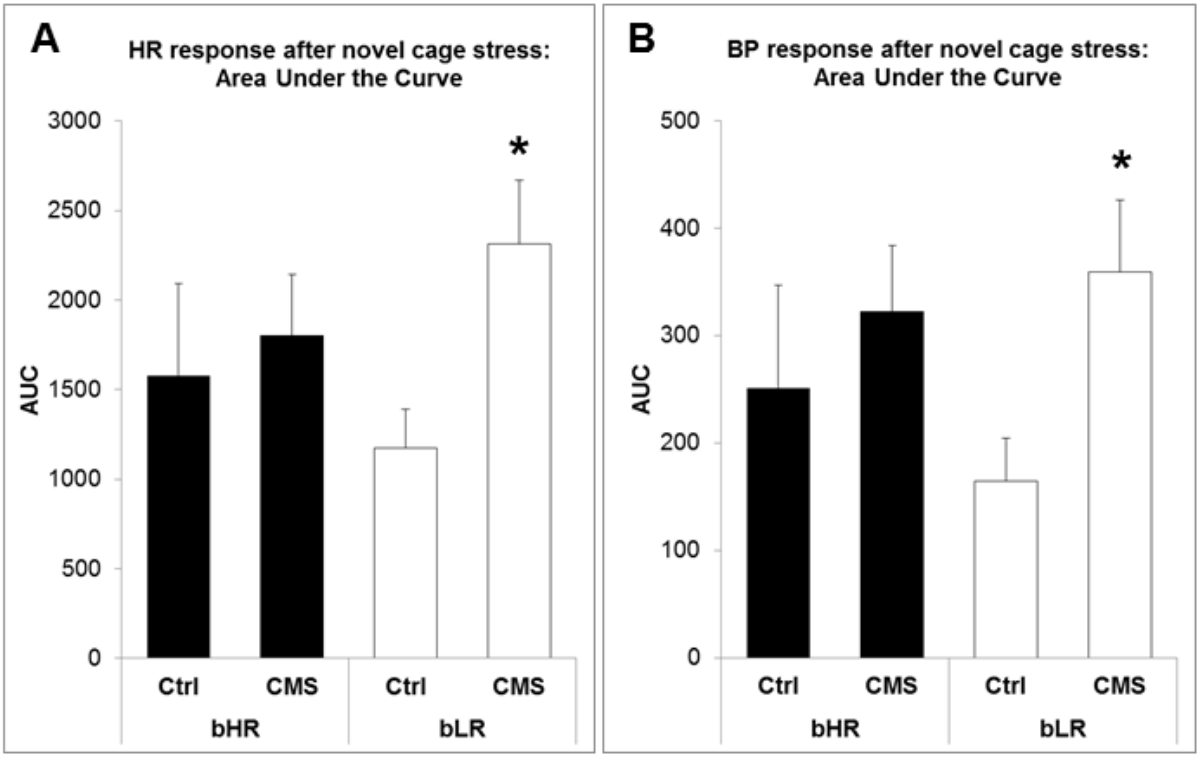


Figure 13: Duration of the increase in HR and BP in response to a novel cage stress

13A: Bar graph of area under the curve (AUC) for heart rate response to cage switch.

13B: Bar graph of area under the curve (AUC) for blood pressure response to cage switch

Values are means ± SEM. n=8/group, except bHR-Control, which had n=7 and two recordings from this group were not usable, thus n=5. *:$p<0.05$ vs. Controls of same strain.

3.5 DISCUSSION

This chapter presents evidence that vulnerability to chronic stress-induced depressive behavior is associated with alterations in cardiovascular function. These changes include increased resting HR, reduced HRV, reduced frequency domain power, and increased cardiovascular reactivity to acute stress. Behavioral data from the rats used in the current study were featured in a previously published report [160] that demonstrated increased vulnerability of bLR rats to CMS-induced depressive behavior compared to bHR rats. These two selectively-bred strains of Sprague Dawley rats were bred based on their low and high locomotor responses to a novel environment, a behavioral phenotype that appears to correspond with similarly divergent behavior in tests of mood and anxiety-like behavior [71, 81]. When bHR and bLR rats were exposed to CMS, bLR-CMS rats showed decreased preference for sucrose compared to bHR-CMS rats, bLR-Control (unexposed) rats, and their own pre-stress baselines beginning in the second week of CMS. This anhedonic behavior persisted throughout the experiment in bLR-CMS rats, but was not observed in bHR-CMS rats until the final week, and even then was less robust, suggesting that bLR and bHR rats provide an excellent model in which to study the interaction of environmental stress and heritable predisposition to depression.

Baseline differences between bLR and bHR rats

The current study expands on previous behavioral findings in bLR and bHR rats by incorporating cardiovascular parameters that were chronically recorded throughout the CMS period. We first

examined whether inherent behavioral differences in locomotion and emotionality in bLR and bHR rats extended to differences in cardiovascular function during the baseline period before CMS began. Though bHR rats tended to have higher HR and BP than bLR rats during both the dark and the light periods, this relationship only reached significance when comparing BP during the dark period. In addition, there were no significant differences in HRV at baseline.

By definition, bHR rats have a higher locomotor response to a novel environment ([71, 81, 123, 124, 160, 161]. This increase in locomotion was also apparent in 24-hour home cage activity, as assessed by telemetry transmitter signal strength. During the baseline period, bHR rats were significantly more active than bLR rats. Interestingly, when cardiovascular reactivity to cage change was measured during Week 5 of CMS, bHR-Control rats tended to have longer BP and HR responses than bHR-CMS rats, as quantified by AUC. Though this difference did not reach statistical significance, bHR rats' extended cardiovascular response may be due in part to their inherent heightened locomotor response to a novel cage, and reduced cardiovascular response in bHR-CMS rats may suggest that CMS blunts the acute locomotor response to novelty, even though 24-hour activity level is not different between strains.

CMS alters cardiovascular function

Previous studies using CMS have found significant changes in cardiovascular function that mirror those observed in human patients with cardiovascular disease [49, 50, 52, 53]. We hypothesized that bLR-CMS rats, which are especially sensitive to depression-like behavior brought on by CMS exposure, would also have an increased sensitivity to CMS-associated cardiovascular changes. Indeed, there was a significant increase in resting HR during the dark period in the bLR-CMS group compared to bHR-CMS and control groups during CMS weeks 3

and 4. No such increase in HR was observed during the light period (0700-1900), and no significant differences were seen in BP during any week, either during the dark or light period. Previous studies have reported a similar elevation in HR recorded during the light period at the end of a four week period of CMS [49, 50, 52, 53] and elevated resting HR is frequently observed in depressed patients throughout the light-dark cycle [164-166].

The CMS model was developed to mimic the types of daily stressors that, over time, result in depression-like signs [46]. The stressors used include changes to the animals' environment, such as overnight lighting and water deprivation, and are assumed to be relatively mild. Despite the frequent use of the CMS model, to our knowledge there has not been a report of the physiological or autonomic impact of individual intermittent mild stressors. Because HR and BP were monitored chronically throughout this experiment, we were able to record cardiovascular responses to many of these stressors, and have analyzed data from strobe light, white noise, and the combination of wet bedding and paired housing. Overall, stressors produced small to moderate increases in BP and HR, with the largest increases being in response to paired housing combined with wet bedding. Though there were no significant differences in peak HR or BP response between bLR and bHR rats, the HR response was prolonged in bLR-CMS rat, as measured by area under the curve once depression-like behaviors have emerged. More typical were the small increases in HR and BP in response to strobe light and loud white noise. bHR and bLR rats did not differ in their response to strobe light turned on during the dark period. bLR rats had higher peak and longer HR responses to white noise during week 2 of CMS, but not during week 4, suggesting that this group may have adapted to the stressor. It is possible that the longer duration of HR response to some stressors in bLR-CMS rats may be indicative of an underlying difference in sensitivity to stress in bLR rats. As cardiovascular reactivity to these

stressors is only one component of autonomic responses to stress, further studies employing measurements of HPA reactivity such as plasma corticosterone or other circulating factors such as cytokines across the 4 weeks of CMS would further clarify this issue.

CMS enhances cardiovascular reactivity to acute stress

The depression-like changes in bLR rats' behavior were also associated with an increase in cardiovascular reactivity to acute stress, as measured by an increase in HR and BP in response to a novel cage. Notably, the stressor used, switching to a clean cage, is a procedure that occurs each week to all rats as their cages are cleaned. Despite the assumed familiarity of this procedure, bLR-CMS rats had a larger increase in HR than either bHR-CMS or control groups. This difference between bLR-CMS and bHR-CMS reactivity appears to be specific to the autonomic regulation of the heart, as both bHR-CMS and bLR-CMS rats had similar BP responses to the cage change, compared to control groups. Additionally, AUC of the HR and BP response to stress was significantly higher in bLR-CMS than bLR-Control rats, and HR and BP remained elevated longer in bLR-CMS rats; however, this relationship was not observed in bHR rats. This increased magnitude of cardiovascular response may be further evidence of the considerable effect of CMS on bLR rats. Baseline BP and HR values just prior to the cage switch test were slightly lower in the rats exposed to CMS, though such differences were not observed when BP or HR were monitored over longer periods of time.

CMS reduces heart rate variability

In addition to the CMS-induced increase in resting HR and heightened cardiovascular reactivity, HRV was significantly reduced in both bHR-CMS and bLR-CMS animals during the fourth

week of CMS. Additionally, HRV was significantly lower in bLR-CMS rats than bHR-CMS rats. Thus, the group that had the greatest degree of anhedonia [160], also had the most reduced HRV. This association of a large reduction in sucrose preference and substantially reduced HRV in bLR rats closely mirrors the correlative relationship between severity of depression and reductions in HRV apparent in depressed patients [21, 33].

Reduced HRV is a critical risk factor for cardiac mortality and likely reflects a disruption in sympathovagal balance to the heart. Reduced HRV in the time domain (s.d. of IBI), can represent an increase in sympathetic activity, a decrease in parasympathetic activity, or both. Frequency domain or spectral analysis of HRV is often used in both experimental and clinical studies as a noninvasive method of assessing autonomic contributions to a global reduction in HRV. Total power across the frequency spectrum is related to overall autonomic balance whereas the HF band corresponds to the respiratory frequency, and is used as an index of vagal or parasympathetic influence on HRV. LF HRV appears to be influenced by both sympathetic and parasympathetic elements [22, 35] and VLF power may reflect cardiovascular modulation by the renin-angiotensin system [29, 35, 36] or its effects on parasympathetic influence on the heart. Frequency domain analysis of HRV during the fourth week of CMS revealed that bLR-CMS rats had reduced total power, HF, and VLF compared to bLR-Control rats ($p<0.05$). Reduced HF power has been observed in both clinical [43] and experimental studies [167] of cardiovascular dysfunction associated with depressive symptoms, and likely reflect a decrease in vagal control of the SA node [168]. Reductions in total power and VLF are most strongly associated with mortality after myocardial infarction, even more so than HF or LF [24], and account for nearly 30% of the risk conferred on cardiac mortality by depression [31], making it one of the most reliable measurements of mortality risk. Taken together, these data suggest that the large

reduction in frequency-domain components of HRV seen in bLR-CMS rats may be due to a decrease in parasympathetic control of the heart mirroring the clinical findings of HRV alterations associated with depression.

Interestingly, this reduction in HRV was not apparent across the entire dark period. For example, when the same HRV analysis was performed during the third quarter of the dark period (0100 – 0400), a period less influenced by changes in the light cycle, there were no significant differences in HRV or its frequency domain components, with the exception of bHR-CMS LF power (**Table 3**). Experimental studies that measure HRV in animals typically do so acutely, upon termination of the experiment, and only for 5- 20 minutes. This is an appropriate analysis method, and, indeed, 20 minutes is the recording length recommended by the Task Force of The European Society of Cardiology and the North American Society of Pacing and Electrophysiology in the prominent article that discusses this method [22]. However, our results suggest that longer recording periods, which may be more comparable to human studies using Holter recordings, likely yield more complex results than short recording periods. Even in the case of animals with extreme anhedonia and greatly reduced HRV, as in the current study, reduced HRV is not apparent at every time period.

Decreased HRV has been reported in various animal models of depression. A series of studies by Grippo et al used CMS to induce anhedonia in rats and demonstrated reduced HRV following 4 weeks of CMS [49, 50, 52, 53, 68]. Similar findings have been reported in studies using social isolation to induce anhedonia in the monogamous prairie vole (for a review, see [158]). After a 4 week period of isolation from their housing partner, both time-domain HRV and HF power (in this case measured by respiratory sinus arrhythmia) were reduced in both male and female prairie voles [167]). HRV was also reduced in the Flinders Sensitive Line (FSL) rat

[169], a strain of rat originally bred for its thermoregulatory sensitivity to cholinergic drugs that has also been shown to be susceptible to depression-like behavior [144]. However, this alteration may be due to physiological changes that are unrelated to the depression-like behavior of the FSL rat, as HRV was also reduced in the Flinders Resistant Line (FRL) rat, and there was no significant difference between FSL and FRL rats. Furthermore, HRV measurements were taken acutely, under urethane anesthesia, which makes them difficult to compare to chronic measures in conscious rats.

In conclusion, vulnerability to CMS-induced depression-like behavior also confers a vulnerability to significant cardiovascular alterations associated with human depression. These include increased resting HR, reduced HRV, changes in frequency domain components of HRV, and increased cardiovascular reactivity. Frequency-domain analysis of HRV suggests that these alterations may be due in part to a decrease in parasympathetic function. This robust model of susceptibility and resistance to CMS-induced depression and associated cardiovascular changes should provide a powerful approach to examining the mechanisms underlying this association and potential therapeutic interventions for treating these serious comorbid conditions.

4.0 CANDESARTAN REVERSES ANHEDONIA AND CARDIOASCULAR ALTERATIONS IN A RODENT MODEL OF DEPRESSION

4.1 ABSTRACT

Major depressive disorder and cardiovascular disease share a comorbid bidirectional relationship, wherein the presence of one increases the likelihood of the other. Antidepressants such as selective serotonin reuptake inhibitors (SSRIs), might not improve cardiovascular mortality, even when depressive signs are ameliorated. Candesartan, an AT1R antagonist often prescribed for hypertension and other cardiovascular disorders, has been shown to be anxiolytic in animal models, and may have antidepressant properties. This study used adult male rats that were selectively-bred for low locomotor response to a novel environment, which are especially vulnerable to chronic mild stress (CMS) -induced signs of depression and associated cardiovascular changes. Rats were exposed to CMS for five weeks, which induced anhedonia and decreased heart rate variability (HRV) and frequency-domain components of HRV, clinically relevant markers of risk for cardiac death; control rats were housed under standard conditions. During the sixth week of the experiment, rats were implanted with subcutaneous osmotic minipumps containing candesartan, the SSRI fluoxetine, or vehicle, and CMS or control housing was continued for an additional four weeks. Candesartan rapidly reversed anhedonic behavior measured by sucrose preference during the first week of treatment. Candesartan also

reduced CMS-induced anxiety-like behavior in the novelty-suppressed feeding test, and had positive effects in the forced swim test in unstressed rats. Furthermore, candesartan reversed the CMS-induced reduction in HRV and its frequency domain components. Although fluoxetine also reversed anhedonic and anxiety-like behavior, and had positive results in the FST, it did not reverse the reduction in HRV and even decreased HRV in control (unstressed) rats. These results provide robust evidence that candesartan is a novel, effective treatment for comorbid depression and cardiovascular disease.

4.2 INTRODUCTION

Major depressive disorder and cardiovascular disease are highly comorbid, and the presence of one disorder increases the likelihood of the other. Depression is an independent risk factor for coronary heart disease both in patients with cardiovascular disease as well as medically healthy individuals [1, 38, 39, 44, 170] and is a significant independent predictor of mortality within 18 months following a heart attack [2, 4]. Frasure-Smith and colleagues [2] found that in the six months following a myocardial infarction, mortality was 3.5 times higher in patients who were also depressed than those who were not.

Animal models of depression have been extremely useful in providing support for this relationship between depression and cardiovascular disease and establishing an experimental model in which to study possible underlying mechanisms and methods of treatment. For instance, the chronic mild stress (CMS) model of depression in rodents, which uses a series of intermittent unpredictable mild stressors to induce an anhedonic state [46, 47], also incorporates many of the cardiovascular disturbances associated with human depression, including increased

resting heart rate (HR) and decreased HR variability (HRV). Decreased HRV is a risk marker for heart attack and cardiovascular mortality [23, 31, 40], and more severe signs of depression are associated with larger reductions in HRV [21, 33].

Recent studies from our lab have shown that rats selectively-bred for reduced locomotor behavior in a novel environment (bred low responder or bLR rats) are especially vulnerable to the anhedonic effects of CMS [160] and that depression-like behaviors in these animals were also associated with changes in cardiovascular function, including decreased HRV, and decreased very low frequency power of HRV, a component of frequency-domain analysis that corresponds to parasympathetic activity and is a predictor of cardiac mortality [3, 31]. Bred high responder (bHR) rats, on the other hand, were resistant to anhedonia, and had only modest reductions in HRV.

There are conflicting reports regarding whether antidepressant therapy improves reduced HRV or risk for cardiac mortality. Tricyclic antidepressants have known cardiotoxic properties and may exacerbate existing cardiovascular conditions [82-84], and their use in patients with cardiovascular disease has been discouraged [85, 86]. While some studies indicate that selective serotonin reuptake inhibitors (SSRIs) improve cardiovascular outcomes [19, 87], others find that SSRIs do not improve mortality risk, even when depression is in remission [88]. Still other studies report that SSRIs may worsen outcomes, especially when administered in conjunction with beta-blockers, which are commonly prescribed for various cardiovascular disorders [89]. In rats exposed to 4 weeks of CMS, simultaneous treatment with the SSRI fluoxetine prevented the anhedonic effects of CMS, but did not fully prevent the reduction in HRV [53].

The renin angiotensin system (RAS) has an established role in cardiovascular regulation, and drugs that inhibit the RAS such as AT1R blockers (ARBs) and angiotensin converting

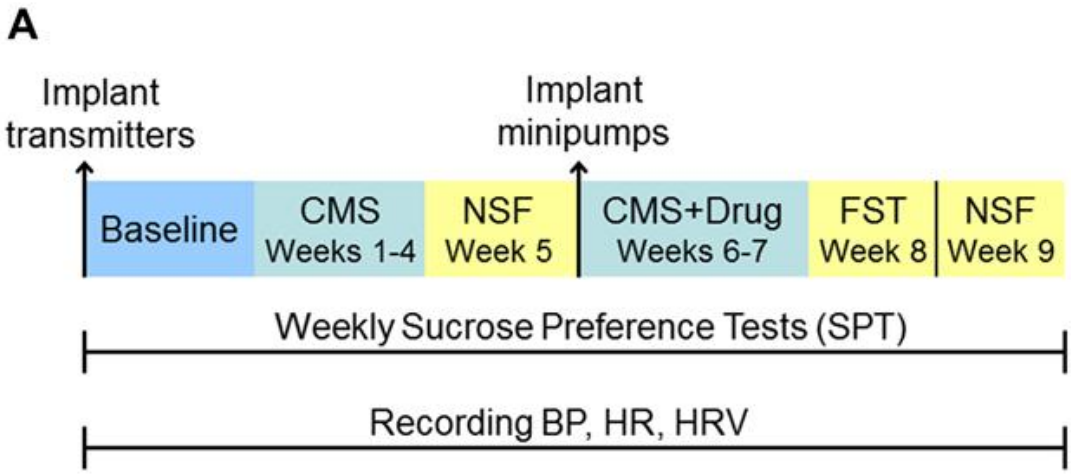
enzyme (ACE) inhibitors have been shown to reduce mortality from heart failure [95], possibly by reversing the reduction in VLF mentioned above [29, 35, 36]. The RAS also plays a key role in the physiological response to acute and chronic stress in studies in rodents. Chronic pretreatment with an ARB reduces anxious behavior in tests such as the elevated plus maze [101], attenuates the hypothalamic pituitary adrenal (HPA) axis response to an acute stress [100] and prevents gastric ulceration associated with a chronic stress [102, 103]. Furthermore, drugs that inhibit the RAS may have antidepressant properties. The ARB losartan and the ACE inhibitor captopril both give positive antidepressant-like effects in the forced swim test in mice [107, 108], a supposed animal model of despair behavior. There is some evidence indicating that ACE inhibitors improve mood and cognitive function in hypertensive patients [104], and antidepressant use is lower in hypertensive patients taking ARBs or ACE inhibitors [106].

The current study investigates the potential antidepressant effects of the ARB candesartan on CMS-induced changes in behavior and cardiovascular function. These experiments were performed in bLR rats, which are especially vulnerable to depression-like behavior [160], and were compared with the effects of the SSRI fluoxetine. We hypothesized that candesartan would reverse CMS-induced anhedonia and the reduction in HRV and its power spectral components, possibly more effectively than fluoxetine.

4.3 EXPERIMENTAL PROCEDURES

Animals and Housing

Adult male bred low responder (bLR) rats were selectively-bred from a Sprague Dawley line at the Molecular and Behavioral Neuroscience Institute at the University of Michigan and shipped to the University of Pittsburgh when they were approximately 2 months old (250-300g). This experiment used 48 bLR rats that were singly housed in plastic bins with wire lids. CMS-exposed and control rats were housed in separate adjacent rooms under similar conditions. The housing rooms were kept at a controlled temperature and humidity on a 12:12 light-dark cycle with lights on at 7:00 am. Food (Purina Chow) and tap water were available ad libitum except where noted as a part of the CMS protocol or testing procedures. Body weight was measured weekly for the duration of the experiment. All animal protocols conform to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh Animal Care and Use Committee. **Figure 14A** shows an outline of the experimental procedures.



B

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Sucrose Preference Test						11a - 12p	
Food deprivation	6p-->	7:30a			5p-->	11a	
Water Deprivation			5p-->	9a	5p-->	11a	
Empty Water Bottle				9a-10a			
Overnight Illumination	7p-->	7a		7p-->	7a		
Cage Tilt		5p-->	9a			12p-4p	
Paired Housing				5p-->	9a		
Damp Bedding	5p-->	9a					
Intermittent White Noise			12p-4p				12p-3p
Strobe Light		2a-6a		1a-4a			
Predator Odor					12p-1p		

Figure 14: Diagram of experimental procedures and typical CMS schedule

Figure 14A: Rats were implanted with telemetry transmitters and allowed 7-10 days to recover. After a baseline recording period, rats were exposed to control conditions or CMS for a total of 9 weeks. Drugs were administered via subcutaneous osmotic minipump beginning in week 6. The novelty-suppressed feeding test (NSF) was administered in weeks 5 and 9 and the forced swim test (FST) was administered in week 8. **Figure 14B**: Rats were exposed to either 9 weeks of intermittent unpredictable stressors or control conditions. Control rats were housed in separate rooms and handled according to usual animal care protocols with the addition of a weekly sucrose preference test (SPT).

Telemetry transmitter implantation surgery

Rats were left undisturbed for one week after arriving at the animal facility at the University of Pittsburgh. After this time, telemetry transmitters (PA-C40, Data Sciences International) were implanted in a subset (n=30) of the rats to record blood pressure (BP), heart rate (HR), and HR variability (HRV); the remaining rats (n=18) were not implanted with transmitters (due to limited availability of transmitters for this experiment). Under isoflurane anesthesia (2% in 100% oxygen, delivered at 1-1.5 L/min), the body of the transmitter was anchored to the abdominal muscle in the intraperitoneal space and the catheter was implanted into the femoral artery. Wounds were closed with silk suture and a single dose of Ketoprofen (2mg/kg, s.c.) was administered as an analgesic. Rats were allowed 7-10 days to recover from surgery before baseline recordings began.

Chronic Mild Stress protocol

Baseline cardiovascular and sucrose preference data (see below) were collected for two weeks before CMS began. Rats were either exposed to CMS (n=24) or were handled according to standard animal care procedures (Control group, n=24) for a total of 9 weeks. The CMS protocol included the following individual stressors, used in varying order across weeks as previously described in detail [160]: continuous overnight lighting; overnight water deprivation (18h) followed by 1 hour of empty water bottle replacement; 40-degree cage tilt; stroboscopic lighting (2-6 h; Chauvet mini-strobe CH-730; 8-12 flashes per second, 35 watts); overnight paired housing with another CMS-exposed rat (18h); damp bedding (300-500mL lukewarm water added to cage bedding); white noise (radio static, 85dB, 1-4 h, continuous or intermittent); and predator odor exposure (30-60 minutes exposure to a small vial of undiluted 2,4,5-

trimethylthiazoline (TMT; Contech Intl.) opened in the housing room). A typical week of stressors is diagrammed in **Figure 14B**.

Sucrose Preference Test

Anhedonia was measured using the sucrose preference test (SPT) as previously described in detail [160]. Rats were accustomed to the taste of sucrose by replacing *ad libitum* water with 1% sucrose for one week. Tap water was returned to the cage for one day, and then rats were food- and water-deprived for 18 hours before the first baseline SPT was administered. During the test, two graduated burets filled with either 1% sucrose solution or tap water were placed on the cage and rats were allowed to drink freely for one hour. Total volume consumed of each fluid was recorded, and preference for sucrose was calculated as [(mL sucrose / total mL consumed) *100]. SPT was administered once per week to both CMS and Control groups (**Figure 14**). Body weight was measured weekly for the duration of the experiment.

Radiotelemetry data collection and analysis

Details of our cardiovascular data collection and analysis methods can be found in the previous chapter. Blood pressure (BP) and heart rate (HR) were recorded as a 10-second average every 2 minutes using Dataquest ART 4.0 software (Data Sciences International). For HRV analysis, pulsatile BP was also recorded from groups of 8 rats at a time on different days of the same week, for 16-24 hours at a time. CMS was continued throughout the recordings.

Time-domain HRV was calculated as the standard deviation of the inter-beat interval (IBI). To evaluate autonomic contributions to HRV, frequency components of HRV were analyzed using the custom open source HRV analysis program, *Physioscripts*, which employs a

band limited variance technique [34] (see Chapter 3). Briefly, the event sequence of IBIs was resampled at 10 Hz, inspected for artifact, and a bandpass filter was applied before calculating the variance within the specified frequency range. Boundaries for the frequency ranges were as follows [163]: total power (TP) 0-5 Hz, high frequency (HF) 1-3 Hz, low frequency (LF) 0.04-1.0 Hz, and very low frequency (VLF) 0-0.39 Hz.

Drug administration

At the beginning of week 6, rats were implanted with osmotic minipumps (Alzet, 2ML4) containing one of the following: candesartan (0.5mg/kg/day; Astra Zeneca), fluoxetine (4mg/kg/day; BioTrend), or 0.9% sterile saline (2mL). These doses were chosen based on previous reports that demonstrated their effects on behavior and neurobiology [66, 100, 101, 171]. Minipumps were loaded and primed for several hours in 37ºC saline and were then surgically implanted subcutaneously in the interscapular region and wounds were closed with silk suture. A single dose of Ketoprofen (2mg/kg, s.c.) was administered as an analgesic. Rats received 3-5 days of drug before the first post-drug SPT was administered (**Figure 14A:** CMS Week 6).

Forced Swim Test

The Forced Swim Test (FST) is a common test of antidepressant drug efficacy, with antidepressant drugs decreasing immobility in this test and increasing time spent in active behavior (i.e., swimming and climbing) [151, 172, 173]. This test was performed during CMS week 8. During swim sessions, rats were placed in a clear Plexiglas cylinder (20cm wide x 61cm high) filled 45 cm high with 23-25ºC tap water. Two sessions were administered: a 15-

minute session to acclimate the rats to the test and apparatus and a 5-minute testing session 24 hours later, which was videotaped for behavioral scoring. Based on the protocol in Cryan et al [151], three behaviors were scored: climbing, during which the rat's front paws move in and out of the water; swimming, in which the rat's front paws remain underwater, while the rat is mobile; and floating or immobility, in which the rat's only movements are to keep its head above the water. The 5-minute testing period was divided into sixty 5-second bins, and the predominant behavior during each bin was scored. Scorers were kept blind to the experimental groups.

Novelty-suppressed feeding test

During Week 5 and Week 9 of CMS, anxiety-like behavior was assessed using the novelty-suppressed feeding test (NSF). Rats were food deprived for 24 hours before being placed in an open field (43cm x 43cm) with a food pellet in the center. Latencies to approach and to consume the food pellet were recorded. Once the rat bit the pellet, or a 15-minute time limit was reached, it was returned to its home cage, and latency to eat in the home cage was measured. Testing took place between 10:00am and 1:00pm.

Data Analysis

Statistical analyses were performed using Microsoft Excel and PASW Statistics 18.0 (SPSS). Values are expressed as mean ± SEM. One-way ANOVA was used for comparisons of CMS and Controls, and for comparisons of drug and treatment groups, a 2x3 ANOVA (Stress x Drug) was used, with Tukey HSD post-hoc tests where appropriate. Repeated measures ANOVA was used to identify significant differences across time, with least square difference (LSD) or Tukey

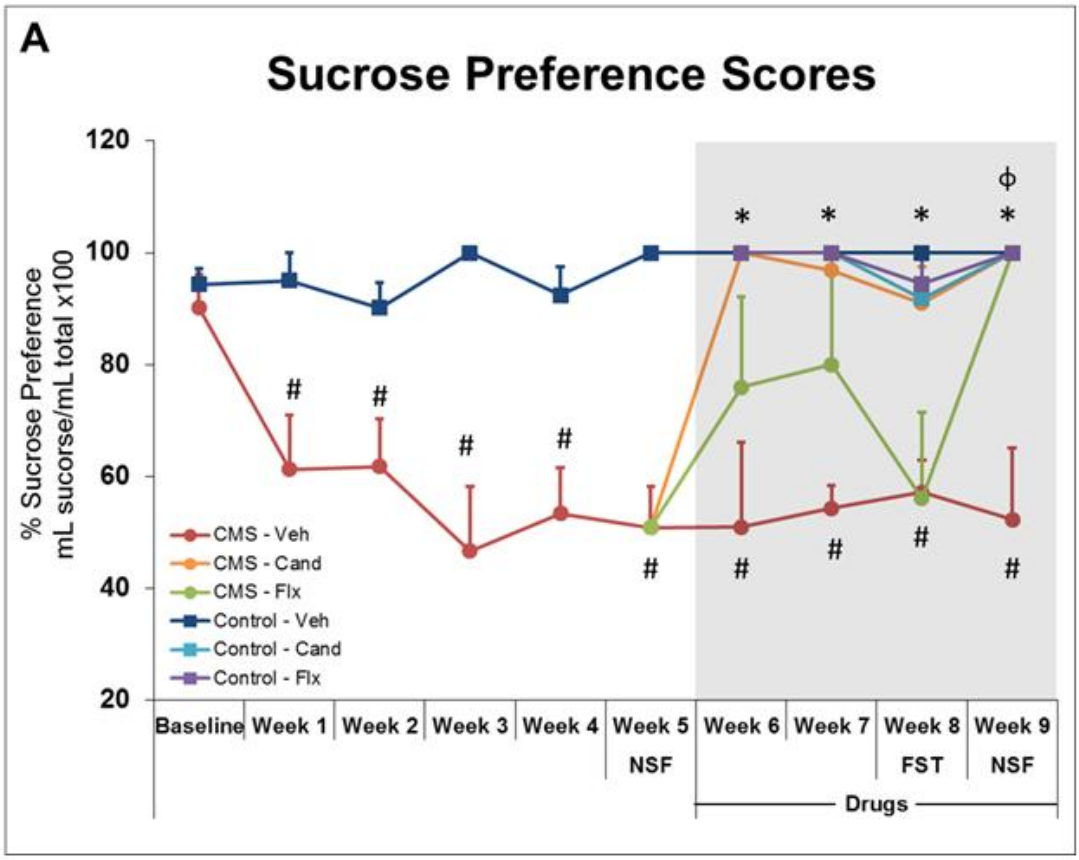
HSD test as appropriate. Pearson's correlation was used to compare HRV and sucrose intake. For all tests, a two-tailed p-value of 0.05 or less was considered significant.

4.4 RESULTS

Sucrose Preference Test

Weekly sucrose preference tests were used to assess anhedonic behavior (**Figure 15A**). At baseline, before CMS began, there was no significant difference between groups ($p=0.48$). By the end of the first week of CMS, the CMS-exposed rats reduced their preference for sucrose by approximately 30% ($p=0.003$), and this persisted through CMS week 5, consistent with our previous studies using bLR rats exposed to CMS [160]. In the beginning of week 6, rats were implanted with osmotic minipumps containing vehicle, candesartan or fluoxetine. When tested at the end of week 6, CMS-exposed rats receiving candesartan had significantly higher sucrose preference scores than those receiving vehicle ($p=0.04$) (**Figure 15B**), and sucrose preference in these rats was not different from either baseline or control rats. The CMS group receiving fluoxetine was not significantly different from CMS-Veh rats during week 6, but was not significantly different from the CMS-candesartan group either, due to the marked variability in the fluoxetine-treated group (**Figure 15B**). After four weeks of drug treatment (CMS week 9), CMS-Cand and CMS-Flx groups were not significantly different from unstressed Controls, though the untreated CMS-Veh group remained significantly anhedonic ($p<0.001$). No significant differences in SPT were observed among Control groups receiving vehicle, fluoxetine or candesartan at any time during the experiment. Body weight, measured weekly throughout

the experiment, did not differ between groups at any time during the experiment (**Figure 16**); across the 9 weeks of the experiment rats typically gained approximately 55 grams.


A
Sucrose Preference Scores
% Sucrose Preference
mL sucorse/mL total x100
120
100
80
60
40
20
CMS - Veh
CMS - Cand
CMS - Flx
Control - Veh
Control - Cand
Control - Flx
Baseline
Week 1
Week 2
Week 3
Week 4
Week 5
NSF
Week 6
Week 7
Week 8
FST
Week 9
NSF
Drugs

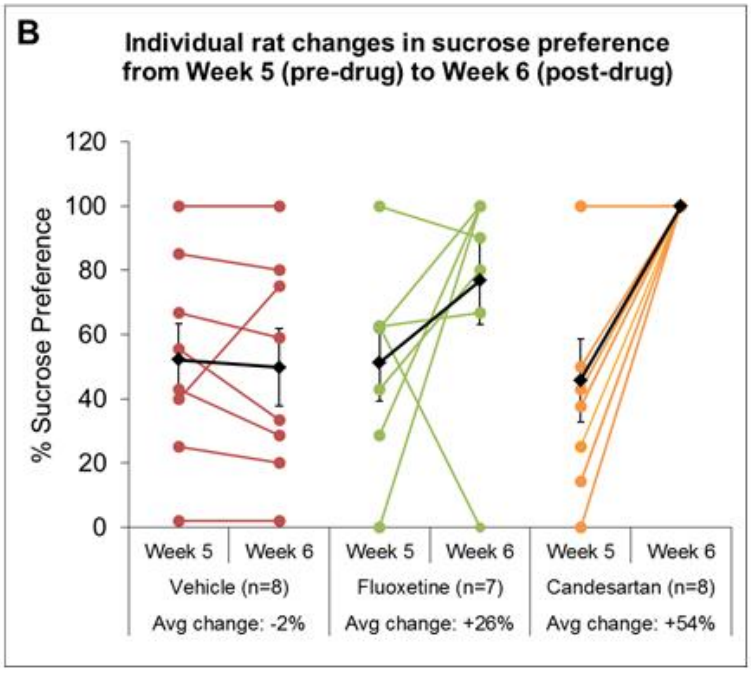

B
Individual rat changes in sucrose preference
from Week 5 (pre-drug) to Week 6 (post-drug)
% Sucrose Preference
120
100
80
60
40
20
0
Week 5
Week 6
Vehicle (n=8)
Avg change: -2%
Week 5
Week 6
Fluoxetine (n=7)
Avg change: +26%
Week 5
Week 6
Candesartan (n=8)
Avg change: +54%

Figure 15: Percent sucrose preference across 9 weeks of CMS

15A: Anhedonia was assessed weekly via SPT. There were no significant differences in sucrose preference at baseline. CMS-exposed rats had significantly lower sucrose preference starting in CMS week 1 and continuing throughout the experiment. Values are means ± SEM. #: $p<0.001$, CMS vs. Control; *: $p<0.01$, CMS-Veh vs. CMS-Cand; ϕ: $p<0.01$, CMS-Veh vs. CMS-Flx. n=8 rats/group, except during week 5, as noted below.

15B: Individual changes in sucrose preference in CMS-exposed rats from CMS week 5 to week 6. Group means are represented in black. Candesartan rapidly increased sucrose preference scores, rats receiving fluoxetine had variable responses, and rats receiving saline did not have a significant change in sucrose preference. Note that one rat from the CMS-Flx group was excluded from this graph because it did not drink during week 5.

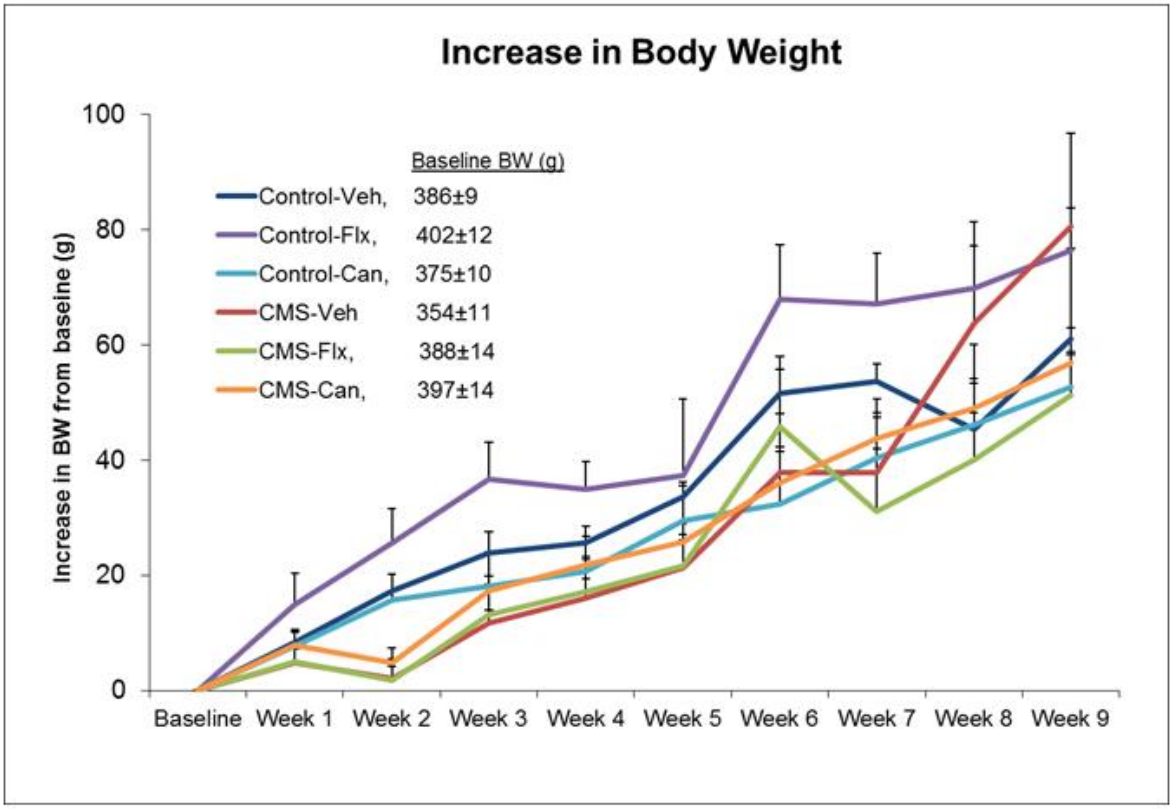


Figure 16: Increase in body weight across 9 weeks of CMS

There were no significant differences among groups in increases in body weight or absolute body weights (not shown) during the experiment. Baseline body weights are listed next to group names in the figure legend. Values are means ± SEM. n=8 rats/group.

Performance in the novelty-suppressed feeding test

The NSF was used to assess anxiety-like behavior. It was performed once in the fifth week of CMS (see **Figure 14A**) to confirm anxiety-like behavior before drugs began [64, 115, 116] and repeated during CMS week 9. At that time, both fluoxetine and candesartan significantly reduced latencies to approach (**Figure 17A**) and eat (**Figure 17B**) the food pellet ($p<0.05$). There were no significant differences among unstressed controls in either of these measures. Among all groups, there were no significant differences in either the number of times rats approached the pellet (**Figure 17C**) or latency to begin eating the in the home cage (**Fig 17D**). These data indicate that candesartan and fluoxetine have similar anxiolytic effects in this model.

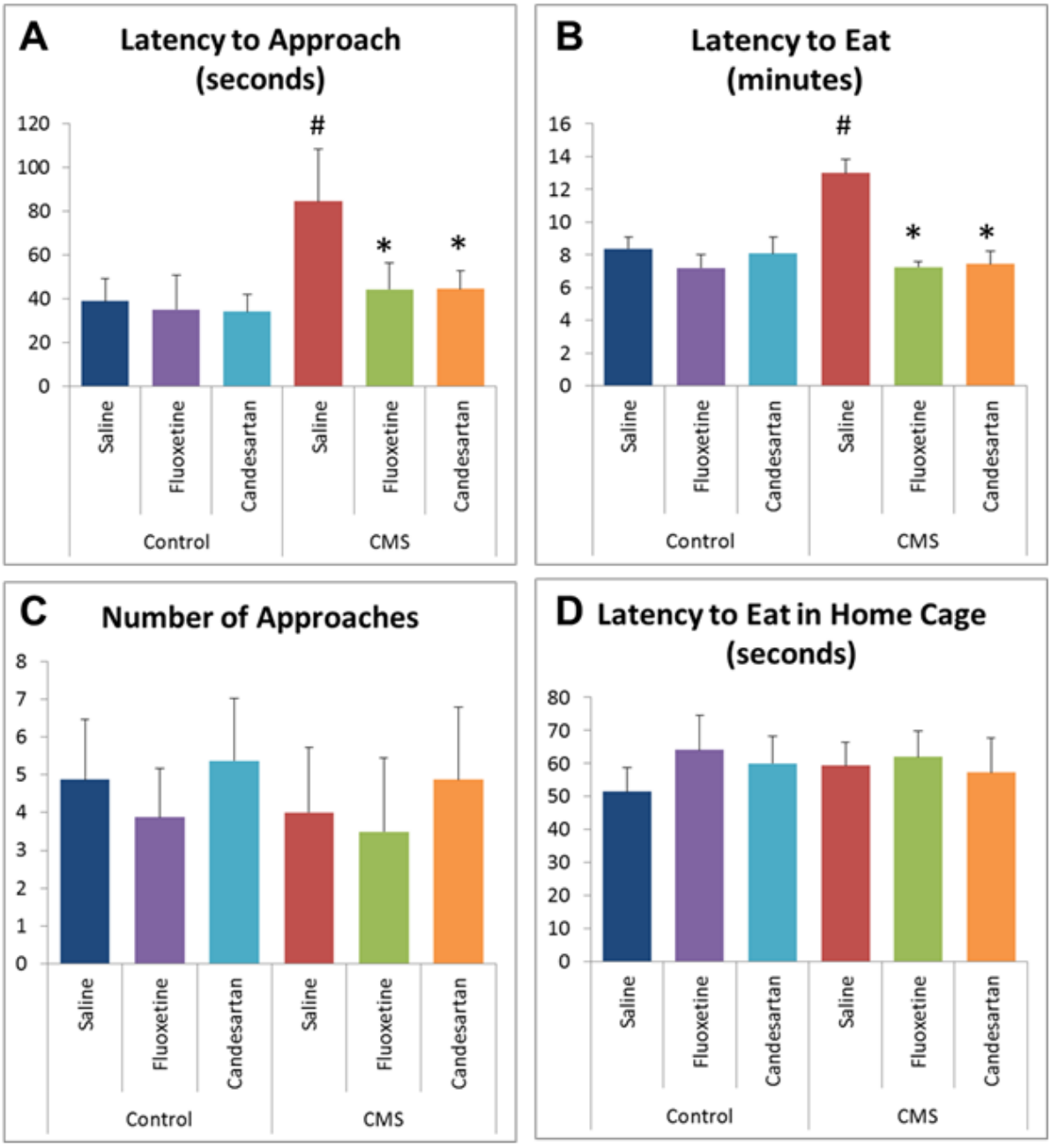


Figure 17: Results from novelty-suppressed feeding test (NSF) test during CMS week 9

Anxiety-like behavior was assessed using the NSF test during the final week of CMS. Candesartan and fluoxetine reduced latencies to approach (**17A**) and eat (**17B**) a food pellet placed in the center of an open field ($p<0.05$). There were no significant differences in the number of times rats approached the food pellet (**17C**) or the latency to eat upon returning to the home cage (**17D**). Values are means ± SEM. *: $p<0.05$ vs. rats receiving vehicle; #:$p<0.05$ vs. control rats receiving the same drug. n=8 rats/group.

Performance in the forced swim test

The FST is frequently used as a predictive test of antidepressant drug efficacy. This test was performed during CMS week 8, after 3 weeks of drug treatment. In unstressed Controls, candesartan significantly decreased time spent immobile ($p=0.04$) and increased swimming behavior ($p=0.05$, **Figure 18A**) but not climbing. Similarly, fluoxetine reduced immobility ($p=0.02$) and increased swimming ($p=0.05$) but not climbing. In the CMS-exposed group, there were no significant differences among treatment groups in immobility, swimming or climbing (**Fig 18B**), but all CMS groups showed decreased immobility and increased swimming compared to control rats treated with vehicle.

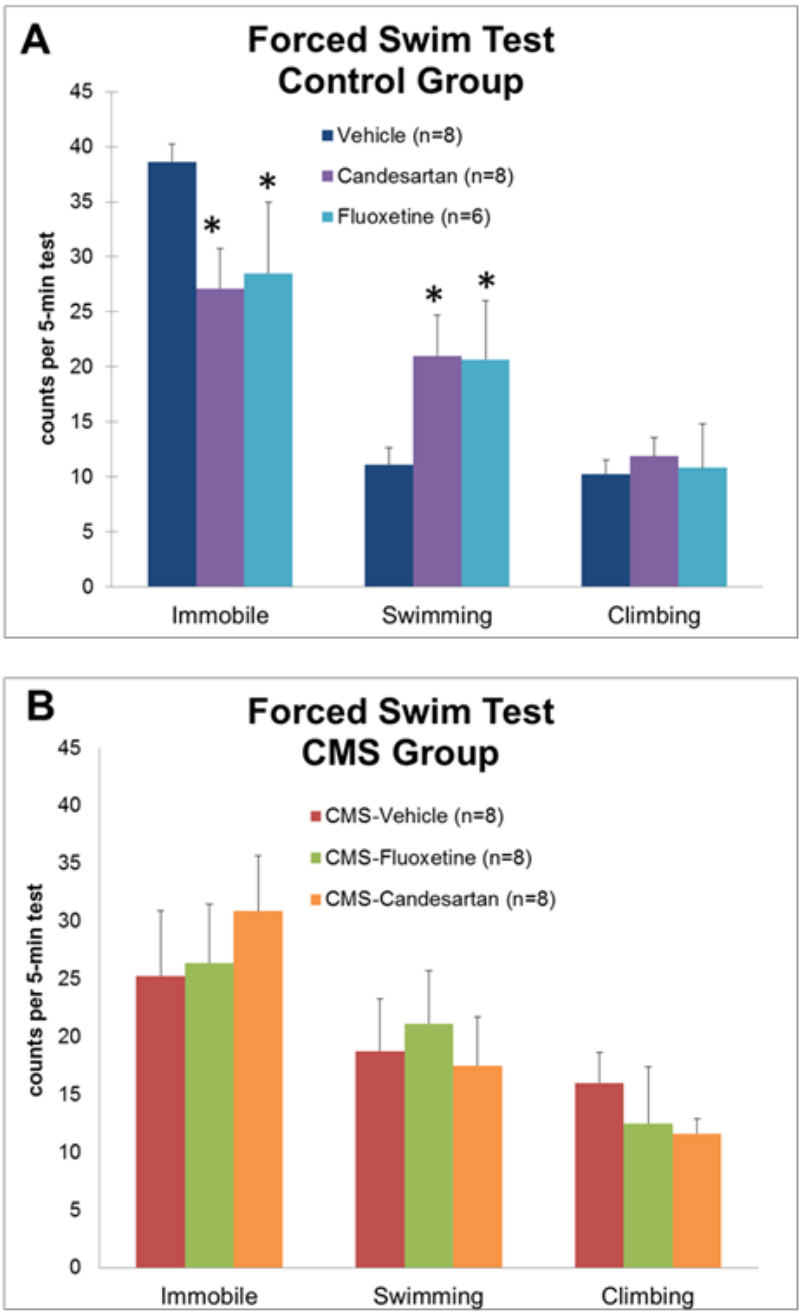
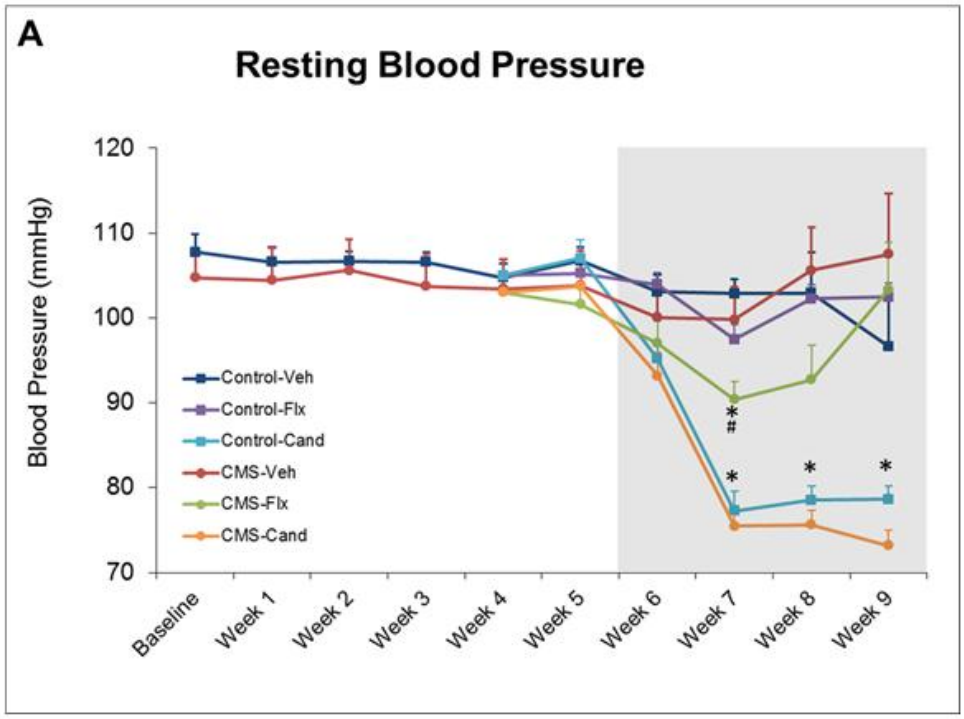


Figure 18: Results from the forced swim test (FST) during CMS week 8

The FST was used to evaluate antidepressant actions of candesartan and fluoxetine. **18A:** In control rats, immobility was decreased by candesartan ($p=0.04$) and fluoxetine ($p=0.02$) and swimming behavior was also increased by both drugs ($p=0.05$ for both)**.** **18B**: In CMS-exposed rats, there were no significant differences among treatment groups. Values are means ± SEM. *: $p<0.05$ vs. rats receiving vehicle. Note that two rats from the Control-Flx group were excluded from analysis because they were large enough to balance on their tails during the FST testing period.

Resting blood pressure and heart rate

HR and BP were chronically recorded throughout the experiment in 5 of the 8 rats in each group. At baseline, there were no significant differences in HR between groups. For the first four weeks of CMS, CMS-exposed rats tended to have higher HR than Control rats, but this difference was not statistically significant (**Figure 19B**). There were also no significant differences in HR once drug administration began. There were no significant differences in BP from baseline through Week 6 of CMS (**Figure 19A)**. During week 7 of CMS, one week after drug treatment had begun, CMS and Control rats receiving candesartan had significantly lower BP compared to rats from the CMS group receiving vehicle ($p<0.005$). Hypotension persisted in these groups for the remainder of the experiment. CMS-Flx rats also had reduced BP during CMS week 7 ($p<0.01$), but BP returned to pre-drug levels during weeks 8 and 9. In addition to analysis of 24-hour averages, light-period and dark-period HR and BP were also analyzed separately, and no significant differences were detected.



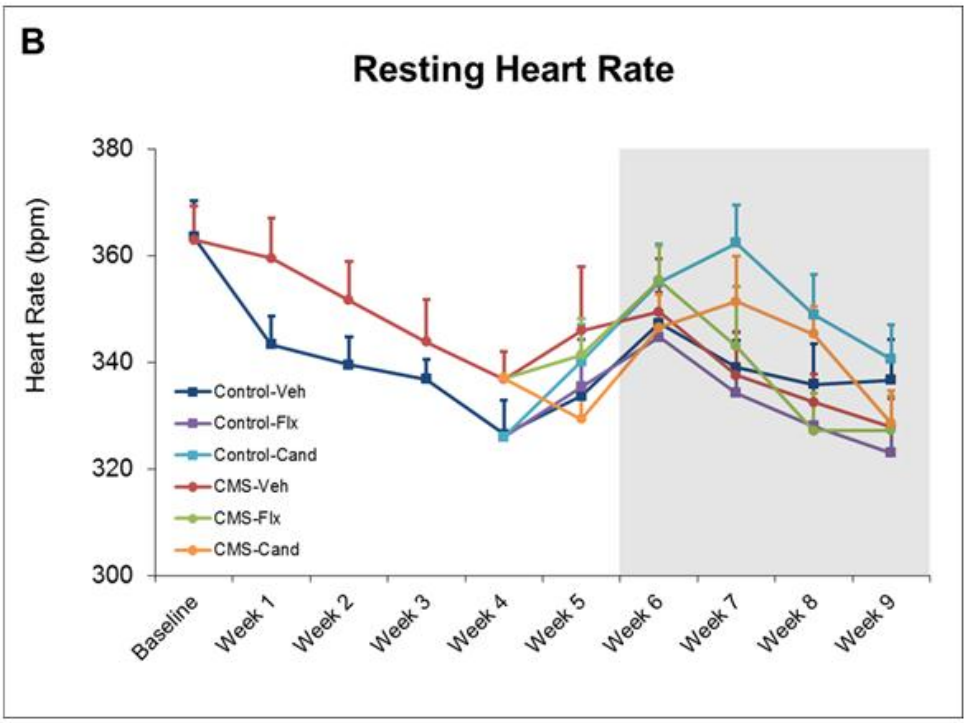


Figure 19: Line graphs of 24-hour resting HR and BP across 9 weeks of CMS

HR and BP were chronically recorded throughout the experiment. Values are means ± SEM. *: $p<0.01$ vs. rats receiving vehicle; #:$p<0.01$ vs. control rats receiving the same drug. **19A:** Candesartan significantly decreased 24-hour resting BP in both CMS-exposed and control rats during CMS weeks 7-9 ($p<0.005$). Fluoxetine also decreased BP in CMS-exposed rats during CMS week 7 ($p<0.01$). **19B:** There were no significant differences in 24-hour resting HR. n=8 rats/group.

Heart Rate Variability

Time domain HRV was analyzed during the second quarter of the dark period (10p-1a) across all weeks of the experiment; this period was chosen because it was common to recordings in all rats during each week. During CMS weeks 1-4, there was a significant effect of CMS ($p=0.01$, **Figure 20A**). CMS reduced HRV during the first week ($p<0.01$), but was not significantly different from controls in weeks 2 and 3. HRV was significantly reduced compared to controls during the fourth and fifth weeks of CMS ($p<0.005$, **Figure 20B**), and remained significantly reduced in CMS-Vehicle rats for the duration of the experiment. HRV was significantly correlated with sucrose intake during week 5 ($R=0.50$, $p=0.005$, $n=48$), the final week of CMS before drugs were administered. HRV was also analyzed during the 3rd quarter of the dark period, but as in the previous study (see Chapter 3) no differences were noted in this later period.

Candesartan, fluoxetine, or vehicle were administered beginning CMS week 6, and there was a significant CMS x Drug interaction in HRV ($p<0.05$) during CMS weeks 7-9. Candesartan reversed the CMS-induced decrease in HRV by week 9 ($p=0.03$ vs. CMS-Veh, **Figure 20B**), and did not significantly change HRV in Control animals. Fluoxetine did not reverse the reduction in HRV in CMS, and during week 9, HRV in CMS-Flx rats was not significantly different CMS-Veh rats (**Figure 20B**). Fluoxetine also significantly decreased HRV in the control group, starting in week 7 ($p=0.05$) and continuing throughout the experiment. As in the weeks before CMS began, HRV remained reduced in CMS-Veh rats compared to Control-Veh rats ($p=0.003$).

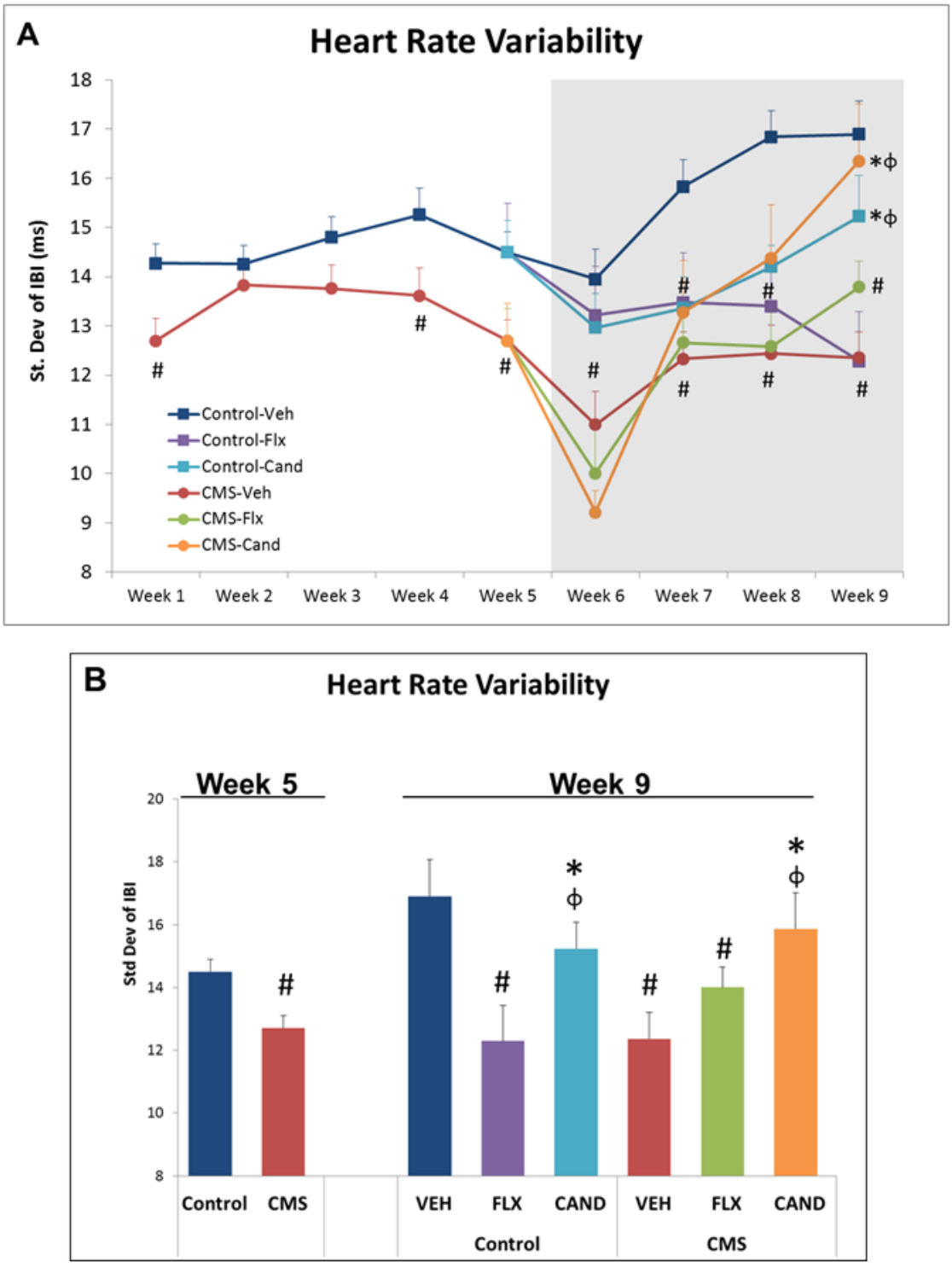


Figure 20: Changes in time-domain HRV across 9 weeks of CMS, with and without drug treatment. HRV was measured during the second quarter of the dark period (11p-1a). **20A**: Line graph of HRV across 9 weeks of CMS. **20B**: Bar graph highlighting HRV during CMS week 5 (week before drug) and CMS week 9 (after 4 weeks of drug). Values are means ± SEM. *:p<0.01 vs. CMS-Veh; #:p<0.05 vs. Control-Veh; ϕ:p<0.05 vs. rats receiving fluoxetine of the same stress group. n=8 rats/group.

Frequency domain analysis of HRV

To evaluate autonomic influences on HRV, frequency domain analysis was performed. During week 5, the final week of CMS before drug treatment began, CMS-exposed rats had a significant decrease in total power ($p=0.003$), VLF power ($p=0.004$), and LF power ($p=0.02$) compared to control rats (data not shown). Sucrose intake was significantly correlated with total power ($R=0.48$, $p=0.008$) and VLF power ($R=0.48$, $p=0.009$) during week 5. By CMS week 9, total power, VLF, HF and LF were still reduced in CMS-Veh rats compared to Control-Veh rats ($p<0.05$) (**Figure 21)**. Candesartan reversed this decrease in each of these frequency bands, and total power, VLF, HF, and LF in CMS-Cand rats were significantly higher than CMS-Veh rats ($p<0.05$). Fluoxetine did not reverse CMS-induced decreases in total power, VLF and LF, and these values were not significantly different from the CMS-Veh group. Fluoxetine also reduced total power, VLF and LF in Control rats, compared to the Control-Veh group ($p<0.05$) and the Control-Cand group ($p<0.05$). HF was not significantly different among Control groups.

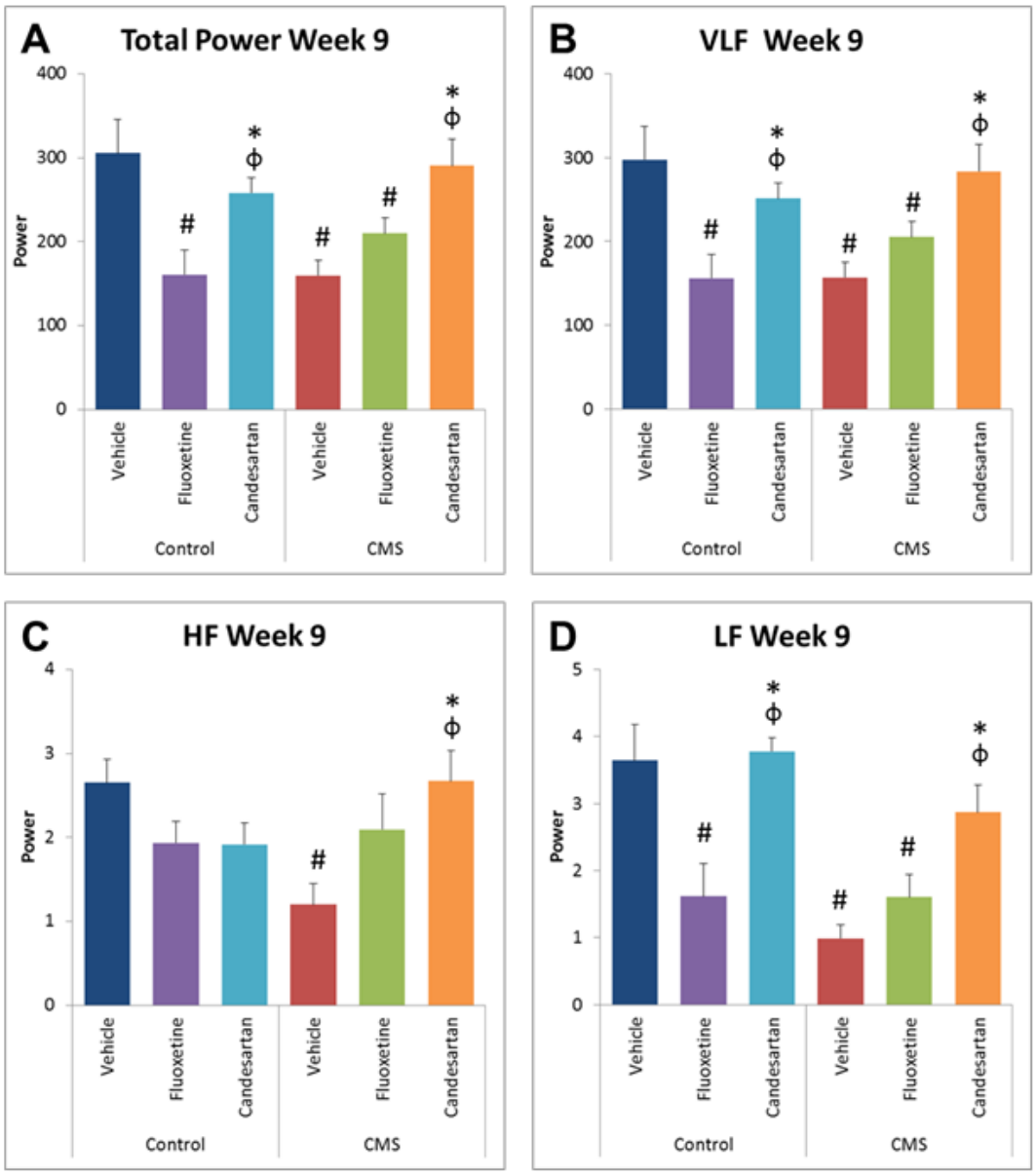


Figure 21: Frequency domain components of HRV during CMS week 9

To assess autonomic contributions to HRV, frequency domain components were also calculated during the second quarter of the dark period. **21A:** Total Power (0-5Hz), **21B:** high frequency power (HF, 1-3Hz), **21C:** low frequency power (LF, 0.04-1.0 Hz), **21D:** very low frequency (VLF, 0-0.39 Hz). Values are means ± SEM. *:p<0.01 vs. CMS-Veh; #:p<0.05 vs. Control-Veh; ϕ:p<0.05 vs. rats receiving fluoxetine of the same stress group. n=8 rats/group.

4.5 DISCUSSION

Cardiovascular disease and depression are highly comorbid. Though the mechanism that connects them is not known, some common physiological, hormonal, and neurotransmitter systems are disrupted in both disorders. One such system is the RAS, which is well-known for its effects on cardiovascular function and may also be involved in altered mood states such as anxiety and depression [100, 101, 108, 171, 174]. The major finding of this study is that candesartan, an ARB used clinically in patients with hypertension or cardiac disease, reverses both the behavioral and the cardiovascular alterations induced by the CMS model of depression. Additionally, the effects of candesartan in our model occur faster and more completely than the SSRI fluoxetine.

CMS induces depression-like effects

bLR rats, which are especially vulnerable to the anhedonic effects of CMS [160], were exposed to 5 weeks of CMS before drug treatment began. CMS induced anhedonia, as measured by preference for a dilute sucrose solution, increased anxiety-like behavior in the NSF test, and reduced HRV and its frequency components. These data replicate previous findings from our lab [160] and others [49-53, 68] regarding the effects of CMS on behavior and cardiovascular function.

Candesartan reverses the behavioral effects of CMS

Drug treatment was started in the beginning of CMS week 6, and rats were tested for anhedonic behavior at the end of the week, 3-5 days after the start of drug administration. In this span of time, candesartan fully reversed anhedonia. Each rat in the CMS-Cand group drank 100%

sucrose within days of receiving candesartan, and this group was not significantly different from control groups on this measure for the remainder of the experiment. Thus, candesartan has robust antidepressant properties in this model. Previous clinical data indicate that patients taking ACE inhibitors have improved mood [104] and those taking ARBs are less likely to be taking antidepressants [106]. This study provides further experimental evidence of the mood-altering properties of ARBs as well as an appropriate model in which to study them.

AT1Rs are located at every level of the HPA axis [98], and blockade with a centrally-acting ARB like candesartan attenuates the HPA response to acute stress [100]. Chronic pretreatment of candesartan greatly reduces anxiety-like behavior in the elevated plus maze in rats, possibly via HPA axis blockade or prevention of the sympathoadrenal response [101]. In the current study, candesartan also had anxiolytic effects in the NSF test. The NSF test has been used frequently in models of depression because chronic, but not acute, antidepressant administration reduces behavioral latencies, providing excellent predictive validity for the time course of antidepressants ([64, 115, 116]). In CMS-exposed rats, candesartan attenuated the latency to approach and to begin to eat the food pellet. Latencies to eat upon return to the home cage were not different among groups, indicating that reduced latencies in the open field are not due to differences in hunger or appetitive value of the food pellet. These results corroborate previous reports of the anxiolytic properties of candesartan in a test with relevance for the effects of antidepressant drugs.

Candesartan reverses CMS-induced reduction in HRV

bLR rats' vulnerability to CMS-induced anhedonia is associated with a decrease in HRV, indicating an imbalance in sympathovagal control of the heart (Chapter 3). Reduced HRV is a

significant marker of risk for cardiac mortality that has been observed in both depression and cardiovascular disease patients [19, 31, 37, 40]. Candesartan reversed the CMS-induced decrease in HRV, restoring it to control (i.e. unstressed) levels. As mentioned in Chapter 3, frequency domain analysis of HRV is a technique that indirectly measures autonomic contributions to HRV. Total power across the frequency spectrum is related to overall autonomic balance, HF power is used as an index of parasympathetic influence on HRV, and LF power appears to be influenced by both sympathetic and parasympathetic elements [22, 35]. Especially pertinent to this study, VLF power may reflect cardiovascular modulation by the renin-angiotensin system [29, 35, 36] or its effects on parasympathetic influence on the heart. In human patients, VLF is considered one of the most reliable indices of risk for cardiac mortality [24], and accounts for nearly 30% of the depression-related risk for heart attack and death [31]. CMS significantly reduced these frequency components compared to unstressed controls, and this reduction was completely reversed by candesartan. Thus, in addition to improving anhedonic behavior within days, candesartan restores sympathovagal balance, measured by HRV and its frequency components.

Fluoxetine reverses the behavioral, but not the cardiovascular effects of CMS

Fluoxetine also reversed CMS-induced anhedonia, though more slowly than candesartan. Individual responses to the initial week of fluoxetine treatment varied, which may parallel the varied responses of depressed patients to the initiation of SSRI treatment [175]. Nonresponse to initial antidepressant treatment is an especially serious issue. Approximately 30% of depression patients do not show improvement after the first course of antidepressant pharmacotherapy [176], and reports suggest that response rates decrease with each subsequent course of treatment

[177]. The weeks-long delay in reversal of anhedonia with SSRIs, also observed in our animal model, is a clinically significant problem; this delay in complete therapeutic efficacy may contribute to patient non-adherence and discontinuation of antidepressant therapy. Therefore, a drug that quickly and robustly improves anhedonia, one of the core signs of depression [12], would be highly beneficial to patient outcomes. Additionally, fluoxetine, like candesartan, reduced anxiety-like behavior in the NSF, as demonstrated in previous reports [64, 115, 116], although this was tested only after 4 weeks of drug treatment so differences in time course for the development of this action were not assessed. The more rapid time course of candesartan versus fluoxetine in reversing CMS-induced anhedonia suggests that these two drugs may be exerting their antidepressant effects via very different mechanisms.

Currently, there is no consensus in the clinical literature as to whether SSRIs reduce the cardiac mortality risk associated with depression, even as they relieve signs of depression [88, 90]. Various studies have shown that SSRIs either improve [19, 87], have no effect on [88], or worsen [89] cardiovascular disease and mortality. A study using the CMS model of depression demonstrated that fluoxetine prevented anhedonia, but not reductions in HRV [53]. We found that fluoxetine did not reverse the CMS-induced decrease in HRV. Though fluoxetine increased HRV slightly in CMS-exposed rats, this increase was not statistically significant from untreated CMS-exposed rats. Fluoxetine also did not improve decreases in the frequency components of HRV. Thus, despite its improvement of the anhedonic and anxiety-like behavior of CMS, we found that fluoxetine does not change the potentially detrimental cardiovascular effects associated with CMS, at least during the time course of this experiment.

Interestingly, fluoxetine administration decreased HRV in control rats to the same degree as CMS exposure. This suggests that fluoxetine may have detrimental cardiovascular effects

independent of its effect on mood, possibly due to interference with normal serotonergic influence of cardiovascular function [178]. Indeed, as mentioned previously, SSRIs can produce cardiovascular disturbance such as arrhythmia in healthy patients without cardiovascular disease [84]. Findings such as these may become increasingly relevant as SSRIs are prescribed for a variety of conditions not directly related to mood [179-181].

Candesartan has antidepressant effects in the forced swim test

The FST is often referred to as a test of learned helplessness [151, 172, 173], operationally defined as an increase in immobility and a decrease in swimming and climbing behaviors. No differences were observed among CMS-exposed rats receiving vehicle or drugs. This is likely an indication of the limited utility of the FST, rather than the effects of CMS on FST behavior. The FST was initially developed to test antidepressant drug efficacy, and not depression-like behavior, per se [172, 182]. When examined in the context of other robust measures of depression-related behaviors, especially the core symptom of anhedonia, these results and past reports suggest that the FST is most reliably employed as an acute test to predict antidepressant drug effects [182, 183].

In Control rats not exposed to CMS, candesartan reduced immobility and increased swimming behavior to the same degree as fluoxetine. Past reports have suggested that antagonists of the serotonergic system increase swimming behavior in the FST, while catecholaminergic antagonists increase climbing behavior [151, 173]. Thus our data suggest that one possible mechanism by which candesartan might be exerting this change is via an interaction with the central serotonergic system [184]. Similar antidepressant effects of drugs that inhibit the RAS have been reported in acute tests of antidepressant efficacy. For instance, the AT1R

antagonist losartan and the ACE inhibitor captopril both give positive anti-depressant like effects in the FST in mice [107, 108]. Previous data from our lab confirm that chronic candesartan also reduces immobility and increases swimming in supplier-bred Sprague Dawley rats [185].

Rapid effects of candesartan suggest a novel antidepressant mechanism

The positive effect of candesartan and fluoxetine in the FST, specifically the increase in swimming behavior, suggests that the two drugs may exert their effects via a similar mechanism. Indeed, there is evidence that the RAS does interact with the serotonergic system [186]. However, the rapid reversal of anhedonia by candesartan suggests that it may be working via a different mechanism. One of the major clinical concerns regarding SSRI treatment is the delay in onset that most patients experience. Though SSRIs such as fluoxetine acutely increase synaptic levels of serotonin, downstream effects such as increases in brain derived neurotrophic factor (BDNF) or increases in hippocampal neurogenesis may be necessary for full antidepressant efficacy [64, 187, 188]. This paradox remains a controversial issue, and the question of the acute versus the long-term mechanisms of SSRI efficacy remains largely unanswered.

Despite candesartan's rapid reversal of anhedonia, HRV is not improved until three weeks later. This suggests that though they are highly comorbid, depression and cardiovascular dysfunction are dissociable. Indeed, in addition to reports of antidepressant effects on HRV, there is evidence that cognitive behavioral therapy improves signs of depression but does not alter cardiac mortality [90]. Therefore, improving the psychological aspects of depression—whether by antidepressant treatment or by psychotherapy—does not necessarily reduce cardiovascular risk.

In addition to interactions with the serotonergic system, there are a number of mechanisms, both direct and indirect, by which candesartan may be reversing anhedonia and reduced HRV. For instance, chronic peripheral administration of candesartan reduces the sympathoadrenal response to stress [189]. The dose and administration of candesartan used in the current study have been shown to have central effects as well [190], and may also be exerting direct effects on central AT1Rs in the hypothalamus or brainstem to alter both cardiovascular and behavioral changes of CMS. Indeed, AT1Rs are co-localized with corticotrophin-releasing hormone (CRH) positive neurons in the PVN [99], and two weeks of pretreatment with candesartan reduces the HPA response to isolation stress [100, 189]. Candesartan also has demonstrated anti-inflammatory effects [191], decreasing, for example, cold-restraint stress-induced increase in gastric ulcers in rats [102], and peripheral and central release of cytokines in response to an endotoxin [192, 193]. Plasma levels of pro-inflammatory cytokines, especially interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-a (TNF-a) are increased in depression and are thought to contribute to some of the somatic symptoms of depression, such as fatigue and reduced appetite [18]. Furthermore, there is evidence that inflammatory markers such as cytokines are correlated with decreased HRV [42, 194, 195], and cytokine levels are increased in chronic heart failure in humans [196]. Indeed, we found that CMS increased plasma levels of IL-1β four-fold in plasma samples collected at the termination of the current experiment, and candesartan attenuated this large increase to control levels (Stedenfeld, Sved & Saavedra, unpublished observations), as did fluoxetine.

Given the distinct latencies of the reversal of behavioral and cardiovascular effects of CMS, candesartan may be affecting these changes via one or more of the above mechanisms. Of course, isolating the precise mechanism is additionally complex because each of the systems

mentioned above—the sympathoadrenal system, the HPA axis, and pro-inflammatory cytokines—also influences the others.

Candesartan is a novel, effective treatment for comorbid depression and cardiovascular disease

Currently, candesartan is frequently prescribed for hypertension and heart failure, is tolerated well with few negative side effects, and has been shown to reduce cardiac mortality [197]. This study presents evidence that candesartan has profound antidepressant effects, including rapid reversal of anhedonia, and attenuated anxiety-like behavior. Furthermore, candesartan reverses the reduced HRV associated with anhedonia, thereby markedly decreasing risk for cardiac mortality. These findings suggest that candesartan and other ARBs may be novel therapies for the treatment of comorbid depression and cardiovascular disease, and may be more effective than traditionally-prescribed antidepressant treatments such as SSRIs.

5.0 GENERAL DISCUSSION

5.1 SUMMARY AND INTERPRETATIONS OF CURRENT FINDINGS

Epidemiological and clinical studies using human patients have provided convincing evidence that depression and cardiovascular disease are highly comorbid [1, 6, 10, 20, 31, 37, 40, 41, 44] and that depression is an independent risk factor for cardiac mortality [2-4, 31], both in patients with existing cardiovascular disease [38, 43] and in medically well individuals [1]. Experimental animal models have been useful in further elucidating mechanisms that may be common to both disorders [49-52, 162]. The studies presented in this dissertation used the CMS model of depression, a well-validated animal model that uses intermittent unpredictable mild stressors to induce anhedonia, one of the core diagnostic criteria for depression [12, 46, 47, 49-52, 68, 198]. CMS also induces a constellation of behavioral, physiological, and neuroendocrine responses that closely resemble those observed in depressed patients [47], including a decrease in HRV, a sign of altered autonomic control of the heart and a marker of cardiac mortality risk [49, 53, 68]. CMS-induced anhedonia is reversed by antidepressant treatment [53, 60, 61], however there is evidence that pretreatment with an SSRI does not improve an anhedonia-associated decrease in HRV, even when depression is improved [53]. This phenomenon has been reported in human patients as well [88, 89], suggesting that in some individuals the cardiac mortality risk associated with depression may persist even when depression is treated.

Many hormonal and neurohumoral systems are activated in both depression and heart disease. One of these systems is the RAS, which has an established role in cardiovascular regulation [91, 92]. Additionally, antagonists of the RAS such as the ARB candesartan greatly reduce the behavioral, neuroendocrine, and sympathoadrenal effects of stress [98, 100, 101, 174, 189], and there is some evidence that ARBs may have antidepressant properties as well [104, 106, 108]. Therefore, the ultimate goal of these experiments was to test the hypothesis that candesartan reverses both the anhedonic and cardiovascular changes induced by CMS, and to compare these results with those of the SSRI fluoxetine.

Preliminary CMS experiments in our lab showed that supplier-bred Sprague Dawley rats had variable responses to four weeks of CMS, and final sucrose preference was positively correlated with pre-CMS locomotor response to an open field (Stedenfeld & Sved, unpublished observation). Indeed, one approach to developing models of genetic predisposition to mood disorders is to selectively-breed animals from within two opposite ends of a behavioral spectrum. We took advantage of one such model, in which rats were selectively-bred based on locomotor response to novelty, bHR and bLR rats (described in detail in Chapter 2). Previously reported differences in mood-related behavior in bHR versus bLR rats [71, 81], led us to hypothesize that the bHR/ bLR distinction might also predict behavioral responses to CMS-exposure. We present these results in **Chapter 2,** providing evidence that bLR rats are especially vulnerable to the anhedonic effects of CMS and also have increased anxiety-like behavior compared to bHR rats. Therefore, the bHR/bLR model appears to be ideal for studying the interactions between inherited vulnerability and environmental stress that may result in depression. At present, little is known about the underlying neurobiology that might contribute to this difference between bHR/bLR rats, and more research in this area is necessary.

We expanded upon these behavioral findings in **Chapter 3** to demonstrate that the respective vulnerability and resistance of bLR and bHR rats to anhedonia is bLR rats had large decreases in HRV and its frequency components, increased resting HR, and increased cardiovascular reactivity to an acute stressor. associated with CMS-induced changes in cardiovascular function that mirror those in depressed patients. These results parallel reports that depressed patients display large decreases in HRV [31] [19], and severity of depression is correlated with reduced HRV [21, 33]. Therefore, the bHR/bLR model is a additionally useful for the study of comorbid depression and cardiovascular disturbances.

It is likely that multiple genes are working together to produce vulnerability to mood disorders such as depression [70]. Models such as the bHR/bLR model take advantage of inborn trait variability to integrate knowledge of behavioral endophenotypes and the genetic and molecular basis of that behavior. As we learn more about how the prenatal environment and early life experience shape the development of neural circuits [150, 199], these types of models will become ever more useful.

Having established a robust model of vulnerability to CMS-induced anhedonia and cardiovascular alterations, in **Chapter 4** we tested the effectiveness of candesartan, an ARB found to reduce cardiovascular mortality in human patients [197], in reversing CMS-induced anhedonia and reduced HRV. Candesartan reversed anhedonia within the first week of treatment, and improved reduced HRV by the fourth week of treatment. It also reduced anxiety-like behavior in CMS-exposed rats and improved scores on the FST in control rats. By contrast, fluoxetine also improved anhedonia, but only after 4 weeks of treatment. Fluoxetine did not improve HRV or its frequency components and, in fact, these measures were decreased in control rats receiving fluoxetine. Thus, candesartan has both robust antidepressant effects and

improves CMS-induced changes in autonomic control of cardiovascular function, and may be more effective than fluoxetine in the treatment of comorbid depression and cardiovascular disease.

Notably, only one dose of each drug was administered in this experiment, via subcutaneous minipump. The dose of candesartan (0.5mg/kg/day, s.c.) was chosen based on reports that this dose and route of administration has effects on central AT1Rs in areas such as the PVN [98, 100, 101, 189, 190]. It is not clear whether peripherally-administered candesartan is accessing the brain by crossing the blood-brain barrier directly or by accessing AT1Rs in circumventricular organs [98]. This dose of candesartan caused a significant hypotension in both CMS-exposed and control rats. However, in control rats, other measures of cardiovascular function, such as 24-hour resting HR, HRV, and frequency components of HRV were not affected by candesartan, suggesting that candesartan is not causing significant changes in overall sympathetic or parasympathetic control of cardiovascular function. Additionally, rats receiving candesartan did not have any noticeable behavioral differences from rats receiving fluoxetine in tests that required physical exertion, such as the FST.

The dose of fluoxetine (4mg/kg/day, s.c.) was chosen based on reports that it attenuated chronic stress-induced CRH expression in the PVN [66]. Using this chronic dose of fluoxetine, we observed a delay in onset of full antidepressant actions that parallels the one seen in depression patients. There were no changes HRV within 4 weeks of fluoxetine treatment, though it is possible that this change may have become apparent with a longer treatment period. For instance, four weeks of pretreatment with fluoxetine (10mg/kg/day, i.p.) partially prevented a CMS-induced decrease in HRV in Sprague Dawley rats [53].

5.2 POSSIBLE MECHANISMS BY WHICH CANDESARTAN REVERSES DEPRESSION-LIKE BEHAVIOR AND CARDIOVASCULAR DYSFUNCTION

Candesartan had temporally distinct effects on behavioral and cardiovascular outcomes. This suggests that though depression and cardiovascular disease have a comorbid, bidirectional relationship, they are dissociable, and can be resolved individually. Thus it is likely that the effects of candesartan are mediated via interactions with one or more of the following physiological systems, all of which play a role in both depression and cardiovascular disease [108, 200].

Inhibition of HPA axis activity

Clinical studies have linked extended hyperactivity of the HPA axis not only with anxiety disorders but also depression [14, 15, 109, 201] . Depressed patients have an increase in HPA activity marked by increased levels of CRH in the cerebrospinal fluid and increased plasma cortisol [17, 202]. In rats, AT1Rs have been found at each level of the HPA axis [98]. They are heavily co-localized with CRH-positive neurons in the parvocellular region of the PVN, and appear to directly modulate their activity [99]. Angiotension stimulation increases CRH synthesis [99] and two weeks of pretreatment with candesartan reduces the HPA response to isolation stress [100, 189]. AT1Rs are also located in the median eminence, the site where CRH is released into the portal circulation, the pituitary, where ACTH is released to the bloodstream, and the adrenal glands, which release corticosterone into the circulation [98].

Although previous studies have found a modest increase in plasma corticosterone following 4 weeks of CMS [51], preliminary results from the current studies do not strongly suggest a central role of altered HPA activity. Indeed, terminal plasma corticosterone levels were not significantly higher in CMS-exposed versus control rats (from Chapter 4), nor were there significant drug effects across groups (Stedenfeld, Sved & Saavedra, unpublished observation). However, CMS is not typically used for longer than 4-6 weeks, and therefore little is known about adaptations that might take place in longer applications. Furthermore, it is possible that acute stress-evoked levels of corticosterone, which were not measured, may be different among groups.

Inhibition of sympathoadrenal responses to CMS

There are significant interactions between the HPA axis and the sympathoadrenal response to stress and depression [203], and sympathoadrenal activity is increased in depression patients and in patients with cardiovascular disease, as marked by increase norepinephrine excretion levels [13]. In rats, AT1Rs are expressed in the adrenal cortex [204], and chronic peripheral administration of candesartan prevents the cold-restraint stress-induced increase in adrenal content of tyrosine hydroxylase mRNA, epinephrine and norepinephrine [91, 189]. Sympathoadrenal activity is also regulated by pre-sympathetic brainstem areas such as the rostral ventrolateral medulla, which is rich in AT1Rs [205, 206].

Effects on hippocampal neurogenesis

There is evidence that antidepressants stimulate adult hippocampal neurogenesis [187, 188] , and that hippocampal neurogenesis may be required for the full therapeutic effects of antidepressants

[64]. Indeed, the latency of therapeutic actions of antidepressants is consistent with the time course of integration of newly adult-born hippocampal neurons [207, 208]. However, though there is some evidence that angiotensin II may have apoptotic effects [209], ARBs do not appear to increase neurogenesis [210-212]. Data from our lab demonstrating that chronic candesartan does not increase the number of hippocampal progenitor cells labeled with 5-bromo-2'-deoxyuridine (BrdU) [185] support this observation.

Anti-inflammatory effects and attenuation of circulating cytokines

Pro-inflammatory cytokines such as TNF-a, IL-6, and IL-1B are increased in depression [18]. Furthermore, cytokine levels are increased in chronic heart failure and following myocardial infarction in humans [196, 213], and there is evidence that inflammatory markers such as cytokines are correlated with decreased HRV [42, 194, 195]. In addition to its direct antagonism of AT1Rs, candesartan has demonstrated anti-inflammatory effects. Candesartan decreases the immune response to an endotoxin [192, 193], is neuroprotective [191, 214], and decreases stress-induced gastric ulcerations [102, 103]. As mentioned in the discussion section of Chapter 4, we found that CMS increased plasma levels of IL-1β four-fold, and candesartan attenuated this large increase to control levels (Stedenfeld, Sved & Saavedra, unpublished observation). Therefore, it is possible that candesartan is having positive effects on anhedonia and cardiovascular function via an anti-inflammatory mechanism. The rapid reversal of anhedonia would support this idea, as a single dose of etanercept, a TNF-a antagonist, was able to decrease chronic heart failure-induced anhedonia to control levels in rats [55].

Antagonists of the RAS also have positive effects on cerebrovascular inflammation, and ARBs such as candesartan have been shown to reduce mortality after ischemic stroke [215],

possibly by increasing cerebral blood flow [216]. These effects have been shown to be independent of effects on AT1Rs, and may instead involve decreasing oxidative stress [217] or restoring endothelial function [191, 218]. There is also evidence that global and regional cerebral blood flow is altered in depression, and antidepressant therapies restore proper blood flow [219, 220]; it has been suggested that increases in cerebral blood flow could act as a biomarker of antidepressant drug effects [221]. It is possible, then, that candesartan is reversing anhedonia via anti-inflammatory effects on the cerebral vasculature that increase cerebral blood flow.

5.3 FUTURE DIRECTIONS

Findings presented in this dissertation provide evidence of a model of increased vulnerability to CMS-induced depression-like behavior and comorbid cardiovascular dysfunction, marked by reduced HRV and frequency domain components of HRV. The bHR/bLR rat model offers an excellent way of studying the genetic and environmental interactions that result in depression-like behavior. However, as previously mentioned, the neurobiological mechanisms that differentiate the bHR and bLR rats are not well-known. Though some information is available from supplier-bred rats that were classified as high- or low-responder rats (e.g. LR rats have increased hippocampal glucocorticoid receptors [76]), not all of these measures have been replicated in bHR/bLR rats. Indeed, these analyses are planned for tissues collected from the current studies.

Furthermore, there is evidence that not all characteristics of the supplier-bred HR/LR classified rats parallel those in selectively-bred bHR/bLR rats. For instance, it has been reported

that when exposed to a repeated social defeat stress, rats classified as HR had greater anxiety-like behavior and reduced sucrose preference compared to unstressed controls [222]. However, there were only very small differences, if any, between HR and LR rats on these measures, further demonstrating that the bHR/bLR model is truly unique in the marked difference in behavior and autonomic cardiovascular function between the two rat strains. Yet this raises the question of how the bHR/bLR rat strains relate to typical Sprague Dawley rats. Do bHR/bLR rats still represent extreme ends of a continuum of normal Sprague Dawley rat behavior, or do they now represent an entirely new rat strain? Exposing supplier-bred HR/LR rats to four weeks of CMS (e.g. replicating Chapters 2 and 3) would be one way to address many of these questions.

This dissertation also presents evidence that the ARB candesartan reverses both anhedonia and changes in cardiovascular function in bLR rats that are vulnerable to these CMS-induced changes. The question of exactly how candesartan is effecting these changes was addressed at length in the previous section and remains an important area of research. Some of these mechanisms will be investigated in future analyses of brains and plasma collected from this experiment. These candidate mechanistic systems are located both centrally and in the periphery, and it is likely that the subcutaneous dose of candesartan we used has effects in both. Answering the question of where candesartan's actions are necessary for its antidepressant effects—in the brain or in the periphery—perhaps by infusing candesartan directly into the ventricles rather than subcutaneously, would be a further step toward understanding the mechanism by which candesartan is working. Finally, if it is determined that candesartan is working via an indirect mechanism, such as by anti-inflammatory actions, it is possible that that system could be a specific target for further antidepressant drug discovery.

5.4 RELEVANCE AND CONCLUSIONS

Cardiovascular disease and major depression are currently two of the most detrimental disorders in developed countries [8, 9]. The comorbid relationship between depression and cardiovascular disease has received attention in recent years as being a serious health concern warranting increased education and awareness in the clinical and health-provider communities [10]. Depression is an independent risk factor for coronary heart disease both in patients with cardiovascular disease as well as medically healthy individuals [1, 38, 39, 44, 170] and is a significant independent predictor of mortality within 18 months following a heart attack [2, 4]. It is alarming, then, that many reports conclude that antidepressant drugs might not improve cardiac mortality, even when they improve depression [19, 53, 84, 90, 223]. Because current antidepressant drugs have a delay in reaching full effect and have varying success rates and tolerability [176, 224], increased research efforts are necessary in antidepressant drug discovery. Furthermore, those efforts should focus on drugs that also address physiological changes that accompany depression. Progress is already being made in this area, as drugs that regulate peptides such as CRH [16], oxytocin [225], vasopressin [226], melatonin [227], and, indeed, the RAS [108] are gaining attention [228].

Furthermore, research into the physiological corollaries of mental disorders has implications beyond finding appropriate pharmacological treatment. Hopefully, as depression is increasingly recognized as a disorder with serious physiological repercussions and not merely a mental disorder, the stigma of such disorders will be further eradicated. There is growing evidence, for example, of improved outreach and treatment for depression and anxiety disorders in vulnerable populations such as postpartum mothers [229, 230] and veterans returning from combat [231, 232]. The experiments described in this dissertation, and future experiments that

examine the relationship between depression and cardiovascular disease, will increase our understanding of the possible mechanisms connecting these two disorders and will encourage more effective treatments in order to improve patients' quality of life.

BIBLIOGRAPHY

[1] Penninx, B. W., Beekman, A. T., Honig, A., Deeg, D. J., Schoevers, R. A., van Eijk, J. T., et al. Depression and cardiac mortality: results from a community-based longitudinal study. Arch Gen Psychiatry. 2001,58:221-7.
[2] Frasure-Smith, N., Lesperance, F., Talajic, M. Depression following myocardial infarction. Impact on 6-month survival. JAMA. 1993,270:1819-25.
[3] Carney, R. M., Blumenthal, J. A., Catellier, D., Freedland, K. E., Berkman, L. F., Watkins, L. L., et al. Depression as a risk factor for mortality after acute myocardial infarction. Am J Cardiol. 2003,92:1277-81.
[4] Frasure-Smith, N., Lesperance, F., Talajic, M. Depression and 18-month prognosis after myocardial infarction. Circulation. 1995,91:999-1005.
[5] Schleifer, S. J., Macari-Hinson, M. M., Coyle, D. A., Slater, W. R., Kahn, M., Gorlin, R., et al. The nature and course of depression following myocardial infarction. Arch Intern Med. 1989,149:1785-9.
[6] Lesperance, F., Frasure-Smith, N., Talajic, M. Major depression before and after myocardial infarction: its nature and consequences. Psychosom Med. 1996,58:99-110.
[7] Glassman, A. H., Shapiro, P. A. Depression and the course of coronary artery disease. Am J Psychiatry. 1998,155:4-11.
[8] Murray, C. J., Lopez, A. D. Evidence-based health policy--lessons from the Global Burden of Disease Study. Science. 1996,274:740-3.
[9] Mathers, C. D., Loncar, D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006,3:e442.
[10] Lichtman, J. H., Bigger, J. T., Jr., Blumenthal, J. A., Frasure-Smith, N., Kaufmann, P. G., Lesperance, F., et al. Depression and coronary heart disease: recommendations for screening, referral, and treatment: a science advisory from the American Heart Association Prevention Committee of the Council on Cardiovascular Nursing, Council on Clinical Cardiology, Council on Epidemiology and Prevention, and Interdisciplinary Council on Quality of Care and Outcomes Research: endorsed by the American Psychiatric Association. Circulation. 2008,118:1768-75.
[11] Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., Monteggia, L. M. Neurobiology of depression. Neuron. 2002,34:13-25.
[12] Association, A. P. Diagnostic and Statistical Manual of Mental Disorders, 4th Edition. Washingtion, DC: American Psychiatric Association; 1994.
[13] Otte, C., Neylan, T. C., Pipkin, S. S., Browner, W. S., Whooley, M. A. Depressive symptoms and 24-hour urinary norepinephrine excretion levels in patients with coronary disease: findings from the Heart and Soul Study. Am J Psychiatry. 2005,162:2139-45.

[14] Holsboer, F. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. J Psychiatr Res. 1999,33:181-214.
[15] Holsboer, F. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology. 2000,23:477-501.
[16] Holsboer, F., Ising, M. Central CRH system in depression and anxiety--evidence from clinical studies with CRH1 receptor antagonists. Eur J Pharmacol. 2008,583:350-7.
[17] Nemeroff, C. B., Widerlov, E., Bissette, G., Walleus, H., Karlsson, I., Eklund, K., et al. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science. 1984,226:1342-4.
[18] Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., Kelley, K. W. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008,9:46-56.
[19] Carney, R. M., Freedland, K. E., Stein, P. K., Skala, J. A., Hoffman, P., Jaffe, A. S. Change in heart rate and heart rate variability during treatment for depression in patients with coronary heart disease. Psychosom Med. 2000,62:639-47.
[20] Carney, R. M., Freedland, K. E., Stein, P. K. Anxiety, depression, and heart rate variability. Psychosom Med. 2000,62:84-7.
[21] Krittayaphong, R., Cascio, W. E., Light, K. C., Sheffield, D., Golden, R. N., Finkel, J. B., et al. Heart rate variability in patients with coronary artery disease: differences in patients with higher and lower depression scores. Psychosom Med. 1997,59:231-5.
[22] Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J. 1996,17:354-81.
[23] Kleiger, R. E., Miller, J. P., Bigger, J. T., Jr., Moss, A. J. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol. 1987,59:256-62.
[24] Bigger, J. T., Jr., Fleiss, J. L., Steinman, R. C., Rolnitzky, L. M., Kleiger, R. E., Rottman, J. N. Frequency domain measures of heart period variability and mortality after myocardial infarction. Circulation. 1992,85:164-71.
[25] Glassman, A. H. Depression, cardiac death, and the central nervous system. Neuropsychobiology. 1998,37:80-3.
[26] Grippo, A. J., Johnson, A. K. Stress, depression and cardiovascular dysregulation: a review of neurobiological mechanisms and the integration of research from preclinical disease models. Stress. 2009,12:1-21.
[27] Gehi, A., Mangano, D., Pipkin, S., Browner, W. S., Whooley, M. A. Depression and heart rate variability in patients with stable coronary heart disease: findings from the Heart and Soul Study. Arch Gen Psychiatry. 2005,62:661-6.
[28] Kop, W. J., Synowski, S. J., Gottlieb, S. S. Depression in heart failure: biobehavioral mechanisms. Heart Fail Clin. 2011,7:23-38.
[29] Bonaduce, D., Marciano, F., Petretta, M., Migaux, M. L., Morgano, G., Bianchi, V., et al. Effects of converting enzyme inhibition on heart period variability in patients with acute myocardial infarction. Circulation. 1994,90:108-13.
[30] Curtis, B. M., O'Keefe, J. H., Jr. Autonomic tone as a cardiovascular risk factor: the dangers of chronic fight or flight. Mayo Clin Proc. 2002,77:45-54.

[31] Carney, R. M., Blumenthal, J. A., Freedland, K. E., Stein, P. K., Howells, W. B., Berkman, L. F., et al. Low heart rate variability and the effect of depression on post-myocardial infarction mortality. Arch Intern Med. 2005,165:1486-91.
[32] Huikuri, H. V., Jokinen, V., Syvanne, M., Nieminen, M. S., Airaksinen, K. E., Ikaheimo, M. J., et al. Heart rate variability and progression of coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 1999,19:1979-85.
[33] Stein, P. K., Domitrovich, P. P., Kleiger, R. E., Schechtman, K. B., Rottman, J. N. Clinical and demographic determinants of heart rate variability in patients post myocardial infarction: insights from the cardiac arrhythmia suppression trial (CAST). Clin Cardiol. 2000,23:187-94.
[34] Allen, J. J., Chambers, A. S., Towers, D. N. The many metrics of cardiac chronotropy: a pragmatic primer and a brief comparison of metrics. Biol Psychol. 2007,74:243-62.
[35] Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A. C., Cohen, R. J. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science. 1981,213:220-2.
[36] Taylor, J. A., Carr, D. L., Myers, C. W., Eckberg, D. L. Mechanisms underlying very-low-frequency RR-interval oscillations in humans. Circulation. 1998,98:547-55.
[37] Carney, R. M., Freedland, K. E., Miller, G. E., Jaffe, A. S. Depression as a risk factor for cardiac mortality and morbidity: a review of potential mechanisms. J Psychosom Res. 2002,53:897-902.
[38] Barefoot, J. C., Helms, M. J., Mark, D. B., Blumenthal, J. A., Califf, R. M., Haney, T. L., et al. Depression and long-term mortality risk in patients with coronary artery disease. Am J Cardiol. 1996,78:613-7.
[39] Barefoot, J. C., Schroll, M. Symptoms of depression, acute myocardial infarction, and total mortality in a community sample. Circulation. 1996,93:1976-80.
[40] Carney, R. M., Freedland, K. E. Depression, mortality, and medical morbidity in patients with coronary heart disease. Biol Psychiatry. 2003,54:241-7.
[41] Frasure-Smith, N., Lesperance, F. Depression and cardiac risk: present status and future directions. Heart. 2010,96:173-6.
[42] Frasure-Smith, N., Lesperance, F., Irwin, M. R., Talajic, M., Pollock, B. G. The relationships among heart rate variability, inflammatory markers and depression in coronary heart disease patients. Brain Behav Immun. 2009,23:1140-7.
[43] Frasure-Smith, N., Lesperance, F., Habra, M., Talajic, M., Khairy, P., Dorian, P., et al. Elevated depression symptoms predict long-term cardiovascular mortality in patients with atrial fibrillation and heart failure. Circulation. 2009,120:134-40, 3p following 40.
[44] Frasure-Smith, N., Lesperance, F. Depression--a cardiac risk factor in search of a treatment. JAMA. 2003,289:3171-3.
[45] Frasure-Smith, N., Lesperance, F. Depression and other psychological risks following myocardial infarction. Arch Gen Psychiatry. 2003,60:627-36.
[46] Willner, P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology. 2005,52:90-110.
[47] Willner, P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology (Berl). 1997,134:319-29.
[48] Parihar, V. K., Hattiangady, B., Kuruba, R., Shuai, B., Shetty, A. K. Predictable chronic mild stress improves mood, hippocampal neurogenesis and memory. Mol Psychiatry. 2011,16:171-83.

[49] Grippo, A. J., Moffitt, J. A., Johnson, A. K. Cardiovascular alterations and autonomic imbalance in an experimental model of depression. Am J Physiol Regul Integr Comp Physiol. 2002,282:R1333-41.
[50] Grippo, A. J., Beltz, T. G., Johnson, A. K. Behavioral and cardiovascular changes in the chronic mild stress model of depression. Physiol Behav. 2003,78:703-10.
[51] Grippo, A. J., Francis, J., Beltz, T. G., Felder, R. B., Johnson, A. K. Neuroendocrine and cytokine profile of chronic mild stress-induced anhedonia. Physiol Behav. 2005,84:697-706.
[52] Grippo, A. J., Sullivan, N. R., Damjanoska, K. J., Crane, J. W., Carrasco, G. A., Shi, J., et al. Chronic mild stress induces behavioral and physiological changes, and may alter serotonin 1A receptor function, in male and cycling female rats. Psychopharmacology (Berl). 2005,179:769-80.
[53] Grippo, A. J., Beltz, T. G., Weiss, R. M., Johnson, A. K. The effects of chronic fluoxetine treatment on chronic mild stress-induced cardiovascular changes and anhedonia. Biol Psychiatry. 2006,59:309-16.
[54] Gambarana, C., Tolu, P. L., Masi, F., Rinaldi, M., Giachetti, D., Morazzoni, P., et al. A study of the antidepressant activity of Hypericum perforatum on animal models. Pharmacopsychiatry. 2001,34 Suppl 1:S42-4.
[55] Grippo, A. J., Francis, J., Weiss, R. M., Felder, R. B., Johnson, A. K. Cytokine mediation of experimental heart failure-induced anhedonia. Am J Physiol Regul Integr Comp Physiol. 2003,284:R666-73.
[56] Di Chiara, G., Tanda, G. Blunting of reactivity of dopamine transmission to palatable food: a biochemical marker of anhedonia in the CMS model? Psychopharmacology (Berl). 1997,134:351-3; discussion 71-7.
[57] Di Chiara, G., Loddo, P., Tanda, G. Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. Biol Psychiatry. 1999,46:1624-33.
[58] Jayatissa, M. N., Bisgaard, C., Tingstrom, A., Papp, M., Wiborg, O. Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. Neuropsychopharmacology. 2006,31:2395-404.
[59] Joeyen-Waldorf, J., Edgar, N., Sibille, E. The roles of sex and serotonin transporter levels in age- and stress-related emotionality in mice. Brain Res. 2009,1286:84-93.
[60] Sampson, D., Willner, P., Muscat, R. Reversal of antidepressant action by dopamine antagonists in an animal model of depression. Psychopharmacology (Berl). 1991,104:491-5.
[61] Muscat, R., Papp, M., Willner, P. Antidepressant-like effects of dopamine agonists in an animal model of depression. Biol Psychiatry. 1992,31:937-46.
[62] Stein, E. J., da Silveira Filho, N. G., Machado, D. C., Hipolide, D. C., Barlow, K., Nobrega, J. N. Chronic mild stress induces widespread decreases in thyroid hormone alpha1 receptor mRNA levels in brain--reversal by imipramine. Psychoneuroendocrinology. 2009,34:281-6.
[63] D'Aquila, P. S., Brain, P., Willner, P. Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. Physiol Behav. 1994,56:861-7.
[64] Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science. 2003,301:805-9.
[65] Czeh, B., Muller-Keuker, J. I., Rygula, R., Abumaria, N., Hiemke, C., Domenici, E., et al. Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. Neuropsychopharmacology. 2007,32:1490-503.

[66] Stout, S. C., Owens, M. J., Nemeroff, C. B. Regulation of corticotropin-releasing factor neuronal systems and hypothalamic-pituitary-adrenal axis activity by stress and chronic antidepressant treatment. J Pharmacol Exp Ther. 2002,300:1085-92.
[67] Grippo, A. J., Moffitt, J. A., Johnson, A. K. Evaluation of baroreceptor reflex function in the chronic mild stress rodent model of depression. Psychosom Med. 2008,70:435-43.
[68] Grippo, A. J., Santos, C. M., Johnson, R. F., Beltz, T. G., Martins, J. B., Felder, R. B., et al. Increased susceptibility to ventricular arrhythmias in a rodent model of experimental depression. Am J Physiol Heart Circ Physiol. 2004,286:H619-26.
[69] Fava, M., Kendler, K. S. Major depressive disorder. Neuron. 2000,28:335-41.
[70] Feder, A., Nestler, E. J., Charney, D. S. Psychobiology and molecular genetics of resilience. Nat Rev Neurosci. 2009,10:446-57.
[71] Stead, J. D., Clinton, S., Neal, C., Schneider, J., Jama, A., Miller, S., et al. Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors. Behav Genet. 2006,36:697-712.
[72] Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell. 2007,131:391-404.
[73] Ballaz, S. J., Akil, H., Watson, S. J. Previous experience affects subsequent anxiety-like responses in rats bred for novelty seeking. Behav Neurosci. 2007,121:1113-8.
[74] Tonissaar, M., Herm, L., Eller, M., Koiv, K., Rinken, A., Harro, J. Rats with high or low sociability are differently affected by chronic variable stress. Neuroscience. 2008,152:867-76.
[75] Touma, C., Bunck, M., Glasl, L., Nussbaumer, M., Palme, R., Stein, H., et al. Mice selected for high versus low stress reactivity: a new animal model for affective disorders. Psychoneuroendocrinology. 2008,33:839-62.
[76] Kabbaj, M., Devine, D. P., Savage, V. R., Akil, H. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. J Neurosci. 2000,20:6983-8.
[77] Mallo, T., Alttoa, A., Koiv, K., Tonissaar, M., Eller, M., Harro, J. Rats with persistently low or high exploratory activity: behaviour in tests of anxiety and depression, and extracellular levels of dopamine. Behav Brain Res. 2007,177:269-81.
[78] Pawlak, C. R., Ho, Y. J., Schwarting, R. K. Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. Neurosci Biobehav Rev. 2008,32:1544-68.
[79] Dellu, F., Piazza, P. V., Mayo, W., Le Moal, M., Simon, H. Novelty-seeking in rats--biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. Neuropsychobiology. 1996,34:136-45.
[80] Jama, A., Cecchi, M., Calvo, N., Watson, S. J., Akil, H. Inter-individual differences in novelty-seeking behavior in rats predict differential responses to desipramine in the forced swim test. Psychopharmacology (Berl). 2008,198:333-40.
[81] Clinton, S. M., Vazquez, D. M., Kabbaj, M., Kabbaj, M. H., Watson, S. J., Akil, H. Individual differences in novelty-seeking and emotional reactivity correlate with variation in maternal behavior. Horm Behav. 2007,51:655-64.
[82] Glassman, A. H., Bigger, J. T., Jr. Cardiovascular effects of therapeutic doses of tricyclic antidepressants. A review. Arch Gen Psychiatry. 1981,38:815-20.
[83] Glassman, A. H., Rodriguez, A. I., Shapiro, P. A. The use of antidepressant drugs in patients with heart disease. J Clin Psychiatry. 1998,59 Suppl 10:16-21.

[84] Sheline, Y. I., Freedland, K. E., Carney, R. M. How safe are serotonin reuptake inhibitors for depression in patients with coronary heart disease? Am J Med. 1997,102:54-9.
[85] Warrington, S. J., Padgham, C., Lader, M. The cardiovascular effects of antidepressants. Psychol Med Monogr Suppl. 1989,16:i-iii, 1-40.
[86] Stern, T. A. The management of depression and anxiety following myocardial infarction. Mt Sinai J Med. 1985,52:623-33.
[87] Glassman, A. H. Does treating post-myocardial infarction depression reduce medical mortality? Arch Gen Psychiatry. 2005,62:711-2.
[88] Jiang, W., Krishnan, R., Kuchibhatla, M., Cuffe, M. S., Martsberger, C., Arias, R. M., et al. Characteristics of depression remission and its relation with cardiovascular outcome among patients with chronic heart failure (from the SADHART-CHF Study). Am J Cardiol. 2011,107:545-51.
[89] Fosbol, E. L., Gislason, G. H., Poulsen, H. E., Hansen, M. L., Folke, F., Schramm, T. K., et al. Prognosis in heart failure and the value of {beta}-blockers are altered by the use of antidepressants and depend on the type of antidepressants used. Circ Heart Fail. 2009,2:582-90.
[90] Berkman, L. F., Blumenthal, J., Burg, M., Carney, R. M., Catellier, D., Cowan, M. J., et al. Effects of treating depression and low perceived social support on clinical events after myocardial infarction: the Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Randomized Trial. JAMA. 2003,289:3106-16.
[91] Jezova, D., Ochedalski, T., Kiss, A., Aguilera, G. Brain angiotensin II modulates sympathoadrenal and hypothalamic pituitary adrenocortical activation during stress. J Neuroendocrinol. 1998,10:67-72.
[92] Saavedra, J. M. Brain angiotensin II: new developments, unanswered questions and therapeutic opportunities. Cell Mol Neurobiol. 2005,25:485-512.
[93] Packer, M. The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. J Am Coll Cardiol. 1992,20:248-54.
[94] Fyhrquist, F., Metsarinne, K., Tikkanen, I. Role of angiotensin II in blood pressure regulation and in the pathophysiology of cardiovascular disorders. J Hum Hypertens. 1995,9 Suppl 5:S19-24.
[95] Ostergren, J. B. Angiotensin receptor blockade with candesartan in heart failure: findings from the Candesartan in Heart failure--assessment of reduction in mortality and morbidity (CHARM) programme. J Hypertens Suppl. 2006,24:S3-7.
[96] Nap, A., Balt, J. C., Mathy, M. J., Van Zwieten, P. A. AT(1)-receptor blockade and sympathetic neurotransmission in cardiovascular disease. Auton Autacoid Pharmacol. 2003,23:285-96.
[97] Saavedra, J. M. Brain and pituitary angiotensin. Endocr Rev. 1992,13:329-80.
[98] Tsutsumi, K., Saavedra, J. M. Characterization and development of angiotensin II receptor subtypes (AT1 and AT2) in rat brain. Am J Physiol. 1991,261:R209-16.
[99] Aguilera, G., Young, W. S., Kiss, A., Bathia, A. Direct regulation of hypothalamic corticotropin-releasing-hormone neurons by angiotensin II. Neuroendocrinology. 1995,61:437-44.
[100] Armando, I., Volpi, S., Aguilera, G., Saavedra, J. M. Angiotensin II AT1 receptor blockade prevents the hypothalamic corticotropin-releasing factor response to isolation stress. Brain Res. 2007,1142:92-9.
[101] Saavedra, J. M., Armando, I., Bregonzio, C., Juorio, A., Macova, M., Pavel, J., et al. A centrally acting, anxiolytic angiotensin II AT1 receptor antagonist prevents the isolation stress-

induced decrease in cortical CRF1 receptor and benzodiazepine binding. Neuropsychopharmacology. 2006,31:1123-34.
[102] Bregonzio, C., Armando, I., Ando, H., Jezova, M., Baiardi, G., Saavedra, J. M. Anti-inflammatory effects of angiotensin II AT1 receptor antagonism prevent stress-induced gastric injury. Am J Physiol Gastrointest Liver Physiol. 2003,285:G414-23.
[103] Bregonzio, C., Armando, I., Ando, H., Jezova, M., Baiardi, G., Saavedra, J. M. Angiotensin II AT1 receptor blockade prevents gastric ulcers during cold-restraint stress. Ann N Y Acad Sci. 2004,1018:351-5.
[104] Germain, L., Chouinard, G. Treatment of recurrent unipolar major depression with captopril. Biol Psychiatry. 1988,23:637-41.
[105] Domeney, A. M. Angiotensin converting enzyme inhibitors as potential cognitive enhancing agents. J Psychiatry Neurosci. 1994,19:46-50.
[106] Nasr, S. J., Crayton, J. W., Agarwal, B., Wendt, B., Kora, R. Lower frequency of antidepressant use in patients on Renin-Angiotensin-aldosterone system modifying medications. Cell Mol Neurobiol. 2011,31:615-8.
[107] Giardina, W. J., Ebert, D. M. Positive effects of captopril in the behavioral despair swim test. Biol Psychiatry. 1989,25:697-702.
[108] Gard, P. R., Mandy, A., Sutcliffe, M. A. Evidence of a possible role of altered angiotensin function in the treatment, but not etiology, of depression. Biol Psychiatry. 1999,45:1030-4.
[109] Kessler, R. C. The effects of stressful life events on depression. Annu Rev Psychol. 1997,48:191-214.
[110] Willner, P., Mitchell, P. J. The validity of animal models of predisposition to depression. Behav Pharmacol. 2002,13:169-88.
[111] Gronli, J., Murison, R., Bjorvatn, B., Sorensen, E., Portas, C. M., Ursin, R. Chronic mild stress affects sucrose intake and sleep in rats. Behav Brain Res. 2004,150:139-47.
[112] Mitchell, P. J. Antidepressant treatment and rodent aggressive behaviour. Eur J Pharmacol. 2005,526:147-62.
[113] Johnson, A. K., Grippo, A. J. Sadness and broken hearts: neurohumoral mechanisms and co-morbidity of ischemic heart disease and psychological depression. J Physiol Pharmacol. 2006,57 Suppl 11:5-29.
[114] Bondi, C. O., Rodriguez, G., Gould, G. G., Frazer, A., Morilak, D. A. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. Neuropsychopharmacology. 2008,33:320-31.
[115] Dulawa, S. C., Hen, R. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. Neurosci Biobehav Rev. 2005,29:771-83.
[116] Bodnoff, S. R., Suranyi-Cadotte, B., Aitken, D. H., Quirion, R., Meaney, M. J. The effects of chronic antidepressant treatment in an animal model of anxiety. Psychopharmacology (Berl). 1988,95:298-302.
[117] Bessa, J. M., Mesquita, A. R., Oliveira, M., Pego, J. M., Cerqueira, J. J., Palha, J. A., et al. A trans-dimensional approach to the behavioral aspects of depression. Front Behav Neurosci. 2009,3:1.
[118] Martinez, V., Rivier, J., Wang, L., Tache, Y. Central injection of a new corticotropin-releasing factor (CRF) antagonist, astressin, blocks CRF- and stress-related alterations of gastric and colonic motor function. J Pharmacol Exp Ther. 1997,280:754-60.
[119] Bonaz, B., Tache, Y. Water-avoidance stress-induced c-fos expression in the rat brain and stimulation of fecal output: role of corticotropin-releasing factor. Brain Res. 1994,641:21-8.

[120] Stam, R., Croiset, G., Akkermans, L. M., Wiegant, V. M. Psychoneurogastroenterology: interrelations in stress-induced colonic motility and behavior. Physiol Behav. 1999,65:679-84.
[121] Cecchi, M., Khoshbouei, H., Javors, M., Morilak, D. A. Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. Neuroscience. 2002,112:13-21.
[122] Hooks, M. S., Jones, G. H., Smith, A. D., Neill, D. B., Justice, J. B., Jr. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse. 1991,9:121-8.
[123] Davis, B. A., Clinton, S. M., Akil, H., Becker, J. B. The effects of novelty-seeking phenotypes and sex differences on acquisition of cocaine self-administration in selectively bred High-Responder and Low-Responder rats. Pharmacol Biochem Behav. 2008,90:331-8.
[124] Flagel, S. B., Robinson, T. E., Clark, J. J., Clinton, S. M., Watson, S. J., Seeman, P., et al. An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: implications for addiction. Neuropsychopharmacology. 2010,35:388-400.
[125] Rush, A. J., Weissenburger, J. E. Melancholic symptom features and DSM-IV. Am J Psychiatry. 1994,151:489-98.
[126] Melartin, T., Leskela, U., Rytsala, H., Sokero, P., Lestela-Mielonen, P., Isometsa, E. Co-morbidity and stability of melancholic features in DSM-IV major depressive disorder. Psychol Med. 2004,34:1443-52.
[127] Loas, G. Vulnerability to depression: a model centered on anhedonia. J Affect Disord. 1996,41:39-53.
[128] Mitchell, P. B., Parker, G. B., Gladstone, G. L., Wilhelm, K., Austin, M. P. Severity of stressful life events in first and subsequent episodes of depression: the relevance of depressive subtype. J Affect Disord. 2003,73:245-52.
[129] Harkness, K. L., Monroe, S. M. Severe melancholic depression is more vulnerable than non-melancholic depression to minor precipitating life events. J Affect Disord. 2006,91:257-63.
[130] Bogdan, R., Pizzagalli, D. A. Acute stress reduces reward responsiveness: implications for depression. Biol Psychiatry. 2006,60:1147-54.
[131] Horesh, N., Klomek, A. B., Apter, A. Stressful life events and major depressive disorders. Psychiatry Res. 2008,160:192-9.
[132] Muscatell, K. A., Slavich, G. M., Monroe, S. M., Gotlib, I. H. Stressful life events, chronic difficulties, and the symptoms of clinical depression. J Nerv Ment Dis. 2009,197:154-60.
[133] Gorwood, P. Neurobiological mechanisms of anhedonia. Dialogues Clin Neurosci. 2008,10:291-9.
[134] Martin-Soelch, C. Is depression associated with dysfunction of the central reward system? Biochem Soc Trans. 2009,37:313-7.
[135] Gold, P. W., Gabry, K. E., Yasuda, M. R., Chrousos, G. P. Divergent endocrine abnormalities in melancholic and atypical depression: clinical and pathophysiologic implications. Endocrinol Metab Clin North Am. 2002,31:37-62, vi.
[136] Gold, P. W., Chrousos, G. P. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Mol Psychiatry. 2002,7:254-75.
[137] Meynen, G., Unmehopa, U. A., van Heerikhuize, J. J., Hofman, M. A., Swaab, D. F., Hoogendijk, W. J. Increased arginine vasopressin mRNA expression in the human hypothalamus in depression: A preliminary report. Biol Psychiatry. 2006,60:892-5.

[138] Kessler, R. C., Gruber, M., Hettema, J. M., Hwang, I., Sampson, N., Yonkers, K. A. Co-morbid major depression and generalized anxiety disorders in the National Comorbidity Survey follow-up. Psychol Med. 2008,38:365-74.
[139] Beesdo, K., Pine, D. S., Lieb, R., Wittchen, H. U. Incidence and risk patterns of anxiety and depressive disorders and categorization of generalized anxiety disorder. Arch Gen Psychiatry. 2010,67:47-57.
[140] Morilak, D. A., Frazer, A. Antidepressants and brain monoaminergic systems: a dimensional approach to understanding their behavioural effects in depression and anxiety disorders. Int J Neuropsychopharmacol. 2004,7:193-218.
[141] Mineka, S., Watson, D., Clark, L. A. Comorbidity of anxiety and unipolar mood disorders. Annu Rev Psychol. 1998,49:377-412.
[142] Regier, D. A., Rae, D. S., Narrow, W. E., Kaelber, C. T., Schatzberg, A. F. Prevalence of anxiety disorders and their comorbidity with mood and addictive disorders. Br J Psychiatry Suppl. 1998:24-8.
[143] Flint, A. J., Rifat, S. L. Two-year outcome of elderly patients with anxious depression. Psychiatry Res. 1997,66:23-31.
[144] Overstreet, D. H., Friedman, E., Mathe, A. A., Yadid, G. The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. Neurosci Biobehav Rev. 2005,29:739-59.
[145] Overstreet, D. H., Pucilowski, O., Djuric, V. Genetic/environment interactions in chronic mild stress. Psychopharmacology (Berl). 1997,134:359-60; discussion 71-7.
[146] Matthews, K., Baldo, B. A., Markou, A., Lown, O., Overstreet, D. H., Koob, G. F. Rewarding electrical brain stimulation: similar thresholds for Flinders Sensitive Line Hypercholinergic and Flinders Resistant Line Hypocholinergic rats. Physiol Behav. 1996,59:1155-62.
[147] Ayensu, W. K., Pucilowski, O., Mason, G. A., Overstreet, D. H., Rezvani, A. H., Janowsky, D. S. Effects of chronic mild stress on serum complement activity, saccharin preference, and corticosterone levels in Flinders lines of rats. Physiol Behav. 1995,57:165-9.
[148] Landgraf, R., Wigger, A. High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. Behav Genet. 2002,32:301-14.
[149] Ohl, F., Toschi, N., Wigger, A., Henniger, M. S., Landgraf, R. Dimensions of emotionality in a rat model of innate anxiety. Behav Neurosci. 2001,115:429-36.
[150] Bosch, O. J., Kromer, S. A., Neumann, I. D. Prenatal stress: opposite effects on anxiety and hypothalamic expression of vasopressin and corticotropin-releasing hormone in rats selectively bred for high and low anxiety. Eur J Neurosci. 2006,23:541-51.
[151] Cryan, J. F., Valentino, R. J., Lucki, I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev. 2005,29:547-69.
[152] Neumann, I. D., Wegener, G., Homberg, J. R., Cohen, H., Slattery, D. A., Zohar, J., et al. Animal models of depression and anxiety: What do they tell us about human condition? Prog Neuropsychopharmacol Biol Psychiatry. 2010.
[153] Veenema, A. H., Torner, L., Blume, A., Beiderbeck, D. I., Neumann, I. D. Low inborn anxiety correlates with high intermale aggression: link to ACTH response and neuronal activation of the hypothalamic paraventricular nucleus. Horm Behav. 2007,51:11-9.
[154] Homberg, J. R., Olivier, J. D., Smits, B. M., Mul, J. D., Mudde, J., Verheul, M., et al. Characterization of the serotonin transporter knockout rat: a selective change in the functioning of the serotonergic system. Neuroscience. 2007,146:1662-76.

[155] Olivier, J. D., Van Der Hart, M. G., Van Swelm, R. P., Dederen, P. J., Homberg, J. R., Cremers, T., et al. A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders. Neuroscience. 2008,152:573-84.
[156] Holmes, A., Yang, R. J., Murphy, D. L., Crawley, J. N. Evaluation of antidepressant-related behavioral responses in mice lacking the serotonin transporter. Neuropsychopharmacology. 2002,27:914-23.
[157] Lira, A., Zhou, M., Castanon, N., Ansorge, M. S., Gordon, J. A., Francis, J. H., et al. Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. Biol Psychiatry. 2003,54:960-71.
[158] Grippo, A. J. Mechanisms underlying altered mood and cardiovascular dysfunction: the value of neurobiological and behavioral research with animal models. Neurosci Biobehav Rev. 2009,33:171-80.
[159] Berntson, G. G., Bigger, J. T., Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology. 1997,34:623-48.
[160] Stedenfeld, K. A., Clinton, S. M., Kerman, I. A., Akil, H., Watson, S. J., Sved, A. F. Novelty-seeking behavior predicts vulnerability in a rodent model of depression. Physiol Behav. 2011,103:210-6.
[161] Clinton, S., Miller, S., Watson, S. J., Akil, H. Prenatal stress does not alter innate novelty-seeking behavioral traits, but differentially affects individual differences in neuroendocrine stress responsivity. Psychoneuroendocrinology. 2008,33:162-77.
[162] Hildreth, C. M., Padley, J. R., Pilowsky, P. M., Goodchild, A. K. Impaired serotonergic regulation of heart rate may underlie reduced baroreflex sensitivity in an animal model of depression. Am J Physiol Heart Circ Physiol. 2008,294:H474-80.
[163] Henze, M., Hart, D., Samarel, A., Barakat, J., Eckert, L., Scrogin, K. Persistent alterations in heart rate variability, baroreflex sensitivity, and anxiety-like behaviors during development of heart failure in the rat. Am J Physiol Heart Circ Physiol. 2008,295:H29-38.
[164] Carney, R. M., Steinmeyer, B., Freedland, K. E., Blumenthal, J. A., Stein, P. K., Steinhoff, W. A., et al. Nighttime heart rate and survival in depressed patients post acute myocardial infarction. Psychosom Med. 2008,70:757-63.
[165] Pitzalis, M. V., Iacoviello, M., Todarello, O., Fioretti, A., Guida, P., Massari, F., et al. Depression but not anxiety influences the autonomic control of heart rate after myocardial infarction. Am Heart J. 2001,141:765-71.
[166] Fox, K., Borer, J. S., Camm, A. J., Danchin, N., Ferrari, R., Lopez Sendon, J. L., et al. Resting heart rate in cardiovascular disease. J Am Coll Cardiol. 2007,50:823-30.
[167] Grippo, A. J., Lamb, D. G., Carter, C. S., Porges, S. W. Social isolation disrupts autonomic regulation of the heart and influences negative affective behaviors. Biol Psychiatry. 2007,62:1162-70.
[168] Japundzic, N., Grichois, M. L., Zitoun, P., Laude, D., Elghozi, J. L. Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. J Auton Nerv Syst. 1990,30:91-100.
[169] Padley, J. R., Overstreet, D. H., Pilowsky, P. M., Goodchild, A. K. Impaired cardiac and sympathetic autonomic control in rats differing in acetylcholine receptor sensitivity. Am J Physiol Heart Circ Physiol. 2005,289:H1985-92.

[170] Anda, R., Williamson, D., Jones, D., Macera, C., Eaker, E., Glassman, A., et al. Depressed affect, hopelessness, and the risk of ischemic heart disease in a cohort of U.S. adults. Epidemiology. 1993,4:285-94.
[171] Pavel, J., Benicky, J., Murakami, Y., Sanchez-Lemus, E., Saavedra, J. M. Peripherally administered angiotensin II AT1 receptor antagonists are anti-stress compounds in vivo. Ann N Y Acad Sci. 2008,1148:360-6.
[172] Porsolt, R. D., Anton, G., Blavet, N., Jalfre, M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol. 1978,47:379-91.
[173] Detke, M. J., Rickels, M., Lucki, I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl). 1995,121:66-72.
[174] Saavedra, J. M., Ando, H., Armando, I., Baiardi, G., Bregonzio, C., Juorio, A., et al. Anti-stress and anti-anxiety effects of centrally acting angiotensin II AT1 receptor antagonists. Regul Pept. 2005,128:227-38.
[175] Charney, D. S., Grothe, D. R., Smith, S. L., Brady, K. T., Kaltsounis-Puckett, J., Wright, C. W., et al. Overview of psychiatric disorders and the role of newer antidepressants. J Clin Psychiatry. 2002,63 Suppl 1:3-9.
[176] Sartorius, N., Baghai, T. C., Baldwin, D. S., Barrett, B., Brand, U., Fleischhacker, W., et al. Antidepressant medications and other treatments of depressive disorders: a CINP Task Force report based on a review of evidence. Int J Neuropsychopharmacol. 2007,10 Suppl 1:S1-207.
[177] Fava, M., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Alpert, J. E., McGrath, P. J., et al. A comparison of mirtazapine and nortriptyline following two consecutive failed medication treatments for depressed outpatients: a STAR*D report. Am J Psychiatry. 2006,163:1161-72.
[178] Ni, W., Watts, S. W. 5-hydroxytryptamine in the cardiovascular system: focus on the serotonin transporter (SERT). Clin Exp Pharmacol Physiol. 2006,33:575-83.
[179] Moja, P. L., Cusi, C., Sterzi, R. R., Canepari, C. Selective serotonin re-uptake inhibitors (SSRIs) for preventing migraine and tension-type headaches. Cochrane Database Syst Rev. 2005:CD002919.
[180] Baldo, P., Doree, C., Lazzarini, R., Molin, P., McFerran, D. J. Antidepressants for patients with tinnitus. Cochrane Database Syst Rev. 2006:CD003853.
[181] Wang, W. F., Chang, L., Minhas, S., Ralph, D. J. Selective serotonin reuptake inhibitors in the treatment of premature ejaculation. Chin Med J (Engl). 2007,120:1000-6.
[182] Borsini, F., Meli, A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl). 1988,94:147-60.
[183] Duman, C. H. Models of depression. Vitam Horm. 2010,82:1-21.
[184] Van de Kar, L. D., Rittenhouse, P. A., Li, Q., Levy, A. D. Serotonergic regulation of renin and prolactin secretion. Behav Brain Res. 1996,73:203-8.
[185] Stedenfeld, K. A., Parrish, J.N. and Sved, A.F. Candesartan, an angiotensin type 1 receptor antagonist, shows positive effects in screening tests of antidepressant drug action The FASEB Journal2011. p. 848.12.
[186] Nalivaiko, E. 5-HT(1A) receptors in stress-induced cardiac changes: a possible link between mental and cardiac disorders. Clin Exp Pharmacol Physiol. 2006,33:1259-64.
[187] Malberg, J. E., Eisch, A. J., Nestler, E. J., Duman, R. S. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci. 2000,20:9104-10.
[188] Duman, R. S. Depression: a case of neuronal life and death? Biol Psychiatry. 2004,56:140-5.

[189] Armando, I., Carranza, A., Nishimura, Y., Hoe, K. L., Barontini, M., Terron, J. A., et al. Peripheral administration of an angiotensin II AT(1) receptor antagonist decreases the hypothalamic-pituitary-adrenal response to isolation Stress. Endocrinology. 2001,142:3880-9.
[190] Nishimura, Y., Ito, T., Hoe, K., Saavedra, J. M. Chronic peripheral administration of the angiotensin II AT(1) receptor antagonist candesartan blocks brain AT(1) receptors. Brain Res. 2000,871:29-38.
[191] Saavedra, J. M., Sanchez-Lemus, E., Benicky, J. Blockade of brain angiotensin II AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia: Therapeutic implications. Psychoneuroendocrinology. 2011,36:1-18.
[192] Benicky, J., Sanchez-Lemus, E., Pavel, J., Saavedra, J. M. Anti-inflammatory effects of angiotensin receptor blockers in the brain and the periphery. Cell Mol Neurobiol. 2009,29:781-92.
[193] Sanchez-Lemus, E., Benicky, J., Pavel, J., Larrayoz, I. M., Zhou, J., Baliova, M., et al. Angiotensin II AT1 blockade reduces the lipopolysaccharide-induced innate immune response in rat spleen. Am J Physiol Regul Integr Comp Physiol. 2009,296:R1376-84.
[194] Ridker, P. M., Rifai, N., Pfeffer, M., Sacks, F., Lepage, S., Braunwald, E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation. 2000,101:2149-53.
[195] Ridker, P. M., Rifai, N., Stampfer, M. J., Hennekens, C. H. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 2000,101:1767-72.
[196] Levine, B., Kalman, J., Mayer, L., Fillit, H. M., Packer, M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med. 1990,323:236-41.
[197] Eklind-Cervenka, M., Benson, L., Dahlstrom, U., Edner, M., Rosenqvist, M., Lund, L. H. Association of candesartan vs losartan with all-cause mortality in patients with heart failure. JAMA. 2011,305:175-82.
[198] Grippo, A. J., Moffitt, J. A., Beltz, T. G., Johnson, A. K. Reduced hedonic behavior and altered cardiovascular function induced by mild sodium depletion in rats. Behav Neurosci. 2006,120:1133-43.
[199] Rinaman, L., Banihashemi, L., Koehnle, T. J. Early life experience shapes the functional organization of stress-responsive visceral circuits. Physiol Behav. 2011.
[200] Grippo, A. J., Johnson, A. K. Biological mechanisms in the relationship between depression and heart disease. Neurosci Biobehav Rev. 2002,26:941-62.
[201] de Kloet, E. R., Joels, M., Holsboer, F. Stress and the brain: from adaptation to disease. Nat Rev Neurosci. 2005,6:463-75.
[202] Banki, C. M., Karmacsi, L., Bissette, G., Nemeroff, C. B. CSF corticotropin-releasing hormone and somatostatin in major depression: response to antidepressant treatment and relapse. Eur Neuropsychopharmacol. 1992,2:107-13.
[203] Brown, M. R., Fisher, L. A., Spiess, J., Rivier, C., Rivier, J., Vale, W. Corticotropin-releasing factor: actions on the sympathetic nervous system and metabolism. Endocrinology. 1982,111:928-31.
[204] Israel, A., Stromberg, C., Tsutsumi, K., Garrido, M. R., Torres, M., Saavedra, J. M. Angiotensin II receptor subtypes and phosphoinositide hydrolysis in rat adrenal medulla. Brain Res Bull. 1995,38:441-6.

[205] Bourassa, E. A., Sved, A. F., Speth, R. C. Anteroposterior distribution of AT(1) angiotensin receptors in caudal brainstem cardiovascular regulatory centers of the rat. Brain Res. 2010,1306:69-76.
[206] Bourassa, E. A., Sved, A. F., Speth, R. C. Angiotensin modulation of rostral ventrolateral medulla (RVLM) in cardiovascular regulation. Mol Cell Endocrinol. 2009,302:167-75.
[207] Esposito, M. S., Piatti, V. C., Laplagne, D. A., Morgenstern, N. A., Ferrari, C. C., Pitossi, F. J., et al. Neuronal differentiation in the adult hippocampus recapitulates embryonic development. J Neurosci. 2005,25:10074-86.
[208] Ngwenya, L. B., Peters, A., Rosene, D. L. Maturational sequence of newly generated neurons in the dentate gyrus of the young adult rhesus monkey. J Comp Neurol. 2006,498:204-16.
[209] Schroder, D., Heger, J., Piper, H. M., Euler, G. Angiotensin II stimulates apoptosis via TGF-beta1 signaling in ventricular cardiomyocytes of rat. J Mol Med. 2006,84:975-83.
[210] Conner, K. R., Payne, V. S., Forbes, M. E., Robbins, M. E., Riddle, D. R. Effects of the AT1 receptor antagonist L-158,809 on microglia and neurogenesis after fractionated whole-brain irradiation. Radiat Res. 2010,173:49-61.
[211] Conner, K. R., Forbes, M. E., Lee, W. H., Lee, Y. W., Riddle, D. AT1 Receptor Antagonism Does Not Influence Early Radiation-Induced Changes in Microglial Activation or Neurogenesis in the Normal Rat Brain. Radiat Res. 2011.
[212] Mukuda, T., Sugiyama, H. An angiotensin II receptor antagonist suppresses running-enhanced hippocampal neurogenesis in rat. Neurosci Res. 2007,58:140-4.
[213] Das, U. N. Free radicals, cytokines and nitric oxide in cardiac failure and myocardial infarction. Mol Cell Biochem. 2000,215:145-52.
[214] Ando, H., Zhou, J., Macova, M., Imboden, H., Saavedra, J. M. Angiotensin II AT1 receptor blockade reverses pathological hypertrophy and inflammation in brain microvessels of spontaneously hypertensive rats. Stroke. 2004,35:1726-31.
[215] Dalmay, F., Mazouz, H., Allard, J., Pesteil, F., Achard, J. M., Fournier, A. Non-AT(1)-receptor-mediated protective effect of angiotensin against acute ischaemic stroke in the gerbil. J Renin Angiotensin Aldosterone Syst. 2001,2:103-6.
[216] Engelhorn, T., Goerike, S., Doerfler, A., Okorn, C., Forsting, M., Heusch, G., et al. The angiotensin II type 1-receptor blocker candesartan increases cerebral blood flow, reduces infarct size, and improves neurologic outcome after transient cerebral ischemia in rats. J Cereb Blood Flow Metab. 2004,24:467-74.
[217] Hamai, M., Iwai, M., Ide, A., Tomochika, H., Tomono, Y., Mogi, M., et al. Comparison of inhibitory action of candesartan and enalapril on brain ischemia through inhibition of oxidative stress. Neuropharmacology. 2006,51:822-8.
[218] Liu, H., Kitazato, K. T., Uno, M., Yagi, K., Kanematsu, Y., Tamura, T., et al. Protective mechanisms of the angiotensin II type 1 receptor blocker candesartan against cerebral ischemia: in-vivo and in-vitro studies. J Hypertens. 2008,26:1435-45.
[219] Ishizaki, J., Yamamoto, H., Takahashi, T., Takeda, M., Yano, M., Mimura, M. Changes in regional cerebral blood flow following antidepressant treatment in late-life depression. Int J Geriatr Psychiatry. 2008,23:805-11.
[220] Kohn, Y., Freedman, N., Lester, H., Krausz, Y., Chisin, R., Lerer, B., et al. 99mTc-HMPAO SPECT study of cerebral perfusion after treatment with medication and electroconvulsive therapy in major depression. J Nucl Med. 2007,48:1273-8.

[221] Chen, Y., Wan, H. I., O'Reardon, J. P., Wang, D. J., Wang, Z., Korczykowski, M., et al. Quantification of cerebral blood flow as biomarker of drug effect: arterial spin labeling phMRI after a single dose of oral citalopram. Clin Pharmacol Ther. 2011,89:251-8.
[222] Duclot, F., Hollis, F., Darcy, M. J., Kabbaj, M. Individual differences in novelty-seeking behavior in rats as a model for psychosocial stress-related mood disorders. Physiol Behav. 2010.
[223] Taylor, C. B., Youngblood, M. E., Catellier, D., Veith, R. C., Carney, R. M., Burg, M. M., et al. Effects of antidepressant medication on morbidity and mortality in depressed patients after myocardial infarction. Arch Gen Psychiatry. 2005,62:792-8.
[224] Moncrieff, J., Kirsch, I. Efficacy of antidepressants in adults. BMJ. 2005,331:155-7.
[225] Grippo, A. J., Trahanas, D. M., Zimmerman, R. R., 2nd, Porges, S. W., Carter, C. S. Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation. Psychoneuroendocrinology. 2009,34:1542-53.
[226] Griebel, G., Simiand, J., Stemmelin, J., Gal, C. S., Steinberg, R. The vasopressin V1b receptor as a therapeutic target in stress-related disorders. Curr Drug Targets CNS Neurol Disord. 2003,2:191-200.
[227] Eser, D., Baghai, T. C., Moller, H. J. Agomelatine: The evidence for its place in the treatment of depression. Core Evid. 2010,4:171-9.
[228] Gurwitz, D., Weizman, A. New drug targets for depression and anxiety: Is the peptides era arriving? Drug Development Research. 2005,65:93-6.
[229] Pinto-Foltz, M. D., Logsdon, M. C. Stigma towards mental illness: a concept analysis using postpartum depression as an exemplar. Issues Ment Health Nurs. 2008,29:21-36.
[230] Goodman, J. H. Women's attitudes, preferences, and perceived barriers to treatment for perinatal depression. Birth. 2009,36:60-9.
[231] Greden, J. F., Valenstein, M., Spinner, J., Blow, A., Gorman, L. A., Dalack, G. W., et al. Buddy-to-Buddy, a citizen soldier peer support program to counteract stigma, PTSD, depression, and suicide. Ann N Y Acad Sci. 2010,1208:90-7.
[232] Olden, M., Cukor, J., Rizzo, A. S., Rothbaum, B., Difede, J. House calls revisited: leveraging technology to overcome obstacles to veteran psychiatric care and improve treatment outcomes. Ann N Y Acad Sci. 2010,1208:133-41.